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The main purpose of the Physiological Reviews is to furnish a means whereby those interested in the physiological sciences may keep in touch with contemporary research. The literature, as every worker knows, is so extensive and scattered that even the specialist may fail to maintain contact with the advance along different lines of his subject. The obvious method of meeting such a situation is to provide articles from time to time in which the more recent literature is compared and summarized. The abstract journals render valuable assistance by condensing and classifying the literature of individual papers, but their function does not extend to a comparative analysis of results and methods. Publications such as the Ergebnisse der Physiologie, the Harvey Lectures, etc., that attempt this latter task, have been so helpful as to encourage the belief that a further enlargement of such agencies will be welcomed by all workers. It is proposed, therefore, to establish a journal in which there will be published a series of short but comprehensive articles dealing with the recent literature in Physiology, using this term in a broad sense to include Bio-chemistry, Bio-physics, Experimental Pharmacology and Experimental Pathology.

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VOL. 55 APRIL 1, 1921 No. 3

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

LV. The Influence of the Vagi on Gastric Tonus and Motility in the Turtle

Z. BERCOVITZ and F. T. ROGERS

From the Hull Physiological Laboratory of the University of Chicago

Received for publication November 6, 1920

The varying results of some preliminary experiments on the action of certain drugs on the gastric medullary centers rendered necessary a more detailed study of the action of the vagus on gastric motility. The extreme ease with which inhibition is induced in mammals suggested that the first experiments be done on animals in which it was thought shock effects were minimal and in which the depressing action of a general anesthetic could be avoided.

As pointed out by Carlson (1), painful stimuli lead to inhibition of gastric motility. Cannon and Murphy (2) have shown that under ether anesthesia the initial passage of food from the stomach was delayed and that the outgo was slow. Meltzer (3) has shown a depression of esophageal peristalsis under anesthesia. In order to avoid the effects both of anesthesia and painful stimuli, resort was had to the common turtle, Chrysemys elegans, in which by transection of the cervical cord it was thought, *a priori*, that one would by such isolation of the brain prevent marked stimulation of gastro-inhibitory centers. Furthermore, such a transection and bilateral vagotomy would allow any necessary dissections below the region of the transection without the use of anesthetics.

It had been noted in the preliminary work (4) that picrotoxin when injected into the cephalic end of the carotid artery caused a spasmodic contraction of the stomach. This was clearly medullary in origin

since it did not appear if the vagi had been sectioned previously. But the stomach contractions induced by picrotoxin soon disappear and immediate injection of more of the drug causes much less effect on the stomach. This seems to be a fatigue effect for if a longer interval be allowed between injections, stomach contractions can be elicited after each injection. Whether this fatigue was central or peripheral was not determined hence it seemed advisable to see if these results could be duplicated by artificial stimulation of the vagus. Among other things it has been found that in the turtle prolonged tetanic contraction of the stomach can not be induced by stimulation of the vagus either through the medullary center with picrotoxin or by electrical stimulation of the vagus trunk. Thus although the heart of the turtle can be held in a state of vagus inhibition for hours, the gastric tetanus breaks down in a few minutes and further vagus stimulation causes no increase in gastric motility.

It was therefore planned to study the effects on gastric motility of various sorts of long continued stimulations, chemical, electrical, mechanical, applied to the cervical vagus trunk. In this report, the effects of electrical stimulation only are given.

Experimental methods: The common turtle, Chrysemys elegans, was used entirely for this study. Two methods of preparation of the animals were employed, the aim of both being to avoid the use of an anesthetic. The first method consisted of making a transverse incision between the first and second cervical vertebrae and completely transecting the spinal cord. The second method consists of decerebrating the animals and is described in detail by Carlson and Luckhardt (5).

The balloon method of recording gastric motility described by Carlson (6) was used. In connecting the balloon with the manometer it was found necessary to use a stiff rubber tube in order to overcome the resistance offered by the bending of the esophagus and also the cardiac sphincter of the stomach. The balloon is inserted into the stomach through the esophagus in the following manner: A transverse incision is made in the esophagus and the rubber tube with the balloon attached is slowly pushed into the stomach. Care must be taken not to tear the esophagus, which is very thin. This part of the operation may require several minutes due to the slowness with which the cardia relaxes. Connections are then made with a water manometer, the writing point of which records on a slowly moving drum. The same water manometer was used throughout the entire series of experiments.

Two types of electrical stimuli were used in these experiments. First induced shocks at the rate of 2400 per minute. Second, induced shocks at the rate of 30 to 40 per minute. The same inductorium was used throughout the entire study. The strength of current is measured as the number of millimeters at which the secondary coil is set from the base of the primary. No strength of current was used which was painful to the tongue.

Artificial respiration was given intermittently in all of the series except the first. All of the experiments are short time acute experiments. The average length of each experiment was 12 to 18 hours, after which the animal was discarded.

Experimental results: After the preparation of the animals and insertion of the balloon in the stomach there is a period of time which ranges from one-half an hour to several hours before there is any indication of gastric motility. Weak contractions appear which in a short time increase in amplitude. There may or may not be an increase in gastric tonus accompanying the increased motility. With an increased gastric tonus, the intra-gastric respiratory variations as recorded may become of less amplitude but are at no time completely obliterated.

It is impossible to predict the motility of the stomach of the turtle by the character of the cord and head reflexes obtained before beginning the experiment. These reflexes are more resistant to traumatic and shock effects than gastric motility. It has been noted in this study that the most active cord and head reflexes are obtained from turtles procured during the summar months, but in these animals there is a condition of diminished gastric tonus after this method of preparation. At no time do these animals develop the degree of gastric motility which the average turtle does that has been obtained during the winter months. It has been further noted that in these turtles which were used during the summer the stomach failed to respond to repeated vagus stimulation much more rapidly than in those animals obtained during the winter months. Section of both vagus nerves causes no changes in gastric tone during the summer. This is directly opposite to the results reported by Hopf (7) who calls special attention to the fact that he was unable to obtain typical results from winter frogs.

There are certain characteristics which follow opening the shell in turtles. These are an apparent increase in gastric tonus indicated by the graphic tracing associated with a depression of the height of the spontaneous contractions (see fig. 1). The pressure of the body cavity of the turtle is less than that of atmospheric pressure. The apparent

increase in gastric tone is not an actual increased intragastric pressure due to increased tonicity of the stomach wall, but is rather an increased extragastric pressure due to putting the body cavity of the turtle under atmospheric pressure. The depression of the amplitude of stomach contractions is also due to the added extra-gastric pressure.

Effects of stimulation of the vagus with rapid induced shocks (2400 per minute): The first series of animals was prepared with the plastron removed completely. Artificial respiration was not given in this series. The cervical vagus or the combined vago-sympathetic nerve was stimulated with a tetanizing current, that is one in which induced shocks were produced at the rate of 2400 per minute.

Fig. 1. Showing the effects on gastric tonus and motility of opening the plastron of the turtle—thus bringing the viscera under atmospheric pressure. *T.P.* = Trephined plastron. Note apparent increased tonus, loss of intragastric respiratory pressure variations, and decreased amplitude of gastric contractions. *R.P.* = Sealed trephine hole and by suction reduced internal pressure to previous level. Balloon in stomach-water manometer. Artificial respiration.

The effects of stimulation of the vago-sympathetic nerve in the turtle with this type of stimulus may be divided into three groups. First, there is a latent period of varying length, depending on the number of times the nerve has been stimulated and also on the condition of the turtle. This varies from 5 to 65 seconds. Second, a relaxation is the first effect on gastric tonus of vago-sympathetic stimulation with rapid induced shocks. This agrees with the work of Hopf (7) on frogs who noted that stimulation of the vagus caused a relaxation of the stomach which was followed by a contraction. This same primary inhibition has been noted in mammals by Cannon (8) who observed that it lasted about 60 seconds and then was followed by a contraction. May (9) and Langely (10) noted the same on their animals. Smith (12), using surviving muscle strips from various parts of the stomach in mammals, observed this inhibitory effect which preceded the contraction from

stimulation of the vagus. On the other hand Openchowski (13) does not appear to have found inhibitory fibers for any portion of the stomach except the cardia, and Goltz (11) working on curarized frogs states that he has never noticed any inhibition of the stomach movements after electrical stimulation of the vagus nerve. These facts indicate a vago-gastric inhibitory mechanism to the musculature of the stomach. Third, there is a contraction of the stomach which follows the two effects described above. The contraction involves the whole stomach. It begins at the cardiac sphincter and travels slowly toward the pyloric end of the stomach. After this constriction has involved the whole stomach relaxation begins at the cardia and progresses over the whole

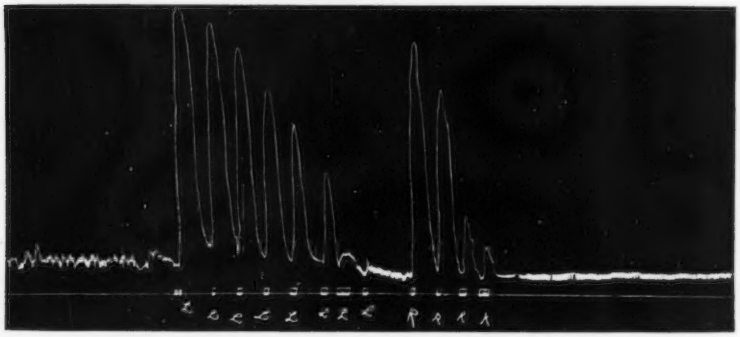

Fig. 2. Gastric contractions following periods of stimulation repeated at intervals of not more than 5 minutes. Rapid induced shocks at the rate of 2400 per minute were used. Artificial respiration. Balloon in stomach, water manometer. *L* = Left vagus nerve stimulated. *R* = Right vagus nerve stimulated.

stomach in the same manner as did the constriction. Relaxation requires a longer time than does contraction. It has been further noted that the amplitude of the contractions following periods of stimulation of uniform intensity repeated at intervals of not more than 5 minutes is lower with each subsequent stimulation (see fig. 2). After each subsequent stimulation there is a fall in intra-gastric tone as well as a depression of the height of the contraction obtained. No difference in the gastric motor effects of stimulating the right and left vagi was observed. These facts are not in accord with the observations of Cannon (14) on dogs; he noted that when the vagus is repeatedly stimulated there results an increased and permanent tone of the stom-

ach. This tonus increases after each stimulation. On the other hand, Doyon (15) observed in the pigeon that if the gizzard be active as the result of stimulation of one vagus it may be stopped by a second stimulation of the same nerve with the same intensity of stimulation.

In the second series of experiments the same procedure as above was carried out in all the animals but in addition they were given artificial

TABLE 1

The effects on gastric contractions of periods of vagus stimulation repeated at time intervals of 5 minutes. Rapid induced shocks and artificial respiration. C = Contraction obtained as the result of vagus stimulation. O = No contraction obtained as the result of vagus stimulation

EXPERIMENT	NERVE	NUMBER OF PERIODS OF STIMULATION OF THE NERVE	EFFECTS OF STIMULATION
I	Left vagus	4	C C C O
II	Left vagus	4	C C C O
	Right vagus	2	C O
III	Left vagus	3	C C O
	Right vagus	2	C O
IV	Left vagus	4	C C C O
	Right vagus	2	C O
V	Left vagus	2	C O
VI	Left vagus	3	C C O
	Right vagus	2	C O
VII	Left vagus	9	C C C C C C C C C O
	Right vagus	4	C C C O

Note: After failure to obtain a contraction from the stimulation of one nerve, the other nerve was then stimulated in the same way. Each successive contraction was lower than the preceding one.

respiration. Seven experiments were performed in this series all of which were similar in their results to those of the first series. The results of this series are given in table 1. When one nerve is repeatedly stimulated with "rapid induced shocks" a condition of depression is brought on in which the stomach fails to contract following stimulation. If after this failure the other nerve be stimulated after the same interval under the same conditions a contraction of the stomach will follow

stimulation of the second nerve, but there is much more rapid depression of the gastric contractions and intra-gastric tone following each subsequent stimulation than there was in the first case.

The factors have not been determined that control the amplitude of the contraction following a given stimulation of the vagus nerve under the conditions of these experiments. In each of the eleven experiments of this series the nerve was stimulated with a given strength of current for a given length of time. In only two cases were the contractions which followed the initial stimulation of the nerve of the same intensity. In all of the other cases the contractions which followed the primary stimulation of the vagus nerve were of different amplitudes ranging from 24 to 140 mm. of water. Also, it is impossible to predict in any given animal how many times stimulation of the vagus may be repeated before the stomach will fail to contract. In five animals the stomach failed to contract after a single stimulation of the vagus while in one case stimulation was repeated seven times. In two cases after failure to obtain a contraction following stimulations repeated at short intervals, the nerves were rested for periods of 90 minutes and 60 minutes respectively. After this interval of rest the nerve was again stimulated and in one case the contraction which followed was 18 mm. lower than the initial contraction and in the other case the contraction was of the same amplitude. After this period of rest in both cases stimulations of the nerve were repeated the same number of times before failure of the contractions occurred. These facts indicate a complete recovery of the stomach after a period of rest. Also, there is either or both a rapid fatiguability of the vago-gastric motor mechanism when the nerve has been repeatedly stimulated with rapid induced shocks, or the development of some kind of a refractory condition within the mechanism. The failure to obtain a gastric motor response as the result of repeated stimulation of the vagus nerve is in marked contrast to the cardiac inhibition that is induced by each stimulation in the same animal. Apparently the vago-cardiac inhibitory mechanism is more resistant to fatigue effects than is the vago-gastric motor mechanism.

There is a striking similarity between this apparent fatigue effect in the vago-gastric mechanism of the turtle and the fatigue of spinal reflexes in mammals as analyzed by Sherrington. There is a progressive decline in reflex activity following repeated stimulation similar to the progressive decrease in the amplitude of the gastric contractions of the turtle following repeated stimulations of the vagus nerve. It has

been further pointed out by Sherrington (25) "that when the scratch reflex elicited from one spot in the skin is fatigued, the fatigue does not implicate the reflex as obtained by stimulation at a distance of two centimeters from the spot fatigued." It is thus to be noted that a contraction of the stomach was obtained from stimulation of the second nerve after failure to obtain a contraction from stimulation of the first nerve.

Effects of stimulation with slow induced shocks, 30 to 40 per minute: In the light of the results obtained from the preceding series, it was suggested by Doctor Carlson that instead of using "rapid induced

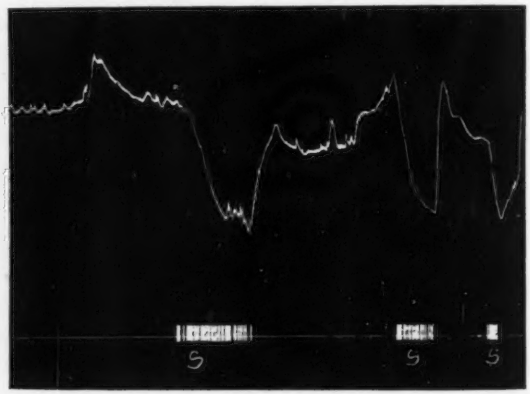

Fig. 3. Effects on gastric tonus of stimulation of the vagus nerve with induced shocks at the rate of 30–40 per minute. Artificial respiration. Balloon in stomach, water manometer. *S* = Stimulated vagus nerve with induced shocks at rate of 30 to 40 per minute.

shocks" for stimulation, single induced shocks at a slow rate be used. All of the turtles in this series were decerebrated and prepared according to the method of Carlson and Luckhardt (5). Artificial respiration was given each animal. The carapace was opened in each case and the stomach dissected free from all of the surrounding membranes. In experiments VII–VIII–IX of this series, the cervical sympathetic nerves were sectioned away from the vagi. Nine experiments were performed in this series. The vagus nerve was stimulated with induced shocks at the rate of 30 to 40 per minute.

In six experiments there was a definite measurable drop in intragastric pressure as the only noticeable result of the stimulation (see fig.

3). In three experiments there was no change observed. In four animals there was also inhibition of the contractions which were spontaneously in progress.

After it was determined that with a given strength of current this effect could be obtained with some degree of certainty, the strength of the current was altered, the rates being kept the same. Various strengths of current were used at the rate of 30 to 40 per minute. In

TABLE 2

Effects of stimulation of the peripheral end of the vagus nerve with slow induced shocks at the rate of 30 to 40 per minute. Shell open, artificial respiration

DATE	EXPERIMENT	INTRA-GASTRIC PRESSURE BEFORE STIMULATION	STRENGTH OF CURRENT USED	INTRA-GASTRIC PRESSURE AFTER STIMULATION WITH SLOW INDUCED SHOCKS-30 TO 40 PER MINUTE
May 1.....	VII	(1) 60 mm. water.		26 mm. water
		(2) 45–60 mm. water		30 mm. water
May 12.....	VIII	(1) 30 mm. water	120	24 mm. water
		(2) 34 mm. water	120	27 mm. water
		(3) 30 mm. water	100	20 mm. water
		(4) 25 mm. water	100	18 mm. water
		(5) 25 mm. water	100	15 mm. water
		(6) 24 mm. water	85	15 mm. water
June 6.....	IX	(1) 32 mm. water	110	24 mm. water
		(2) 29 mm. water	110	18 mm. water
		(3) 34 mm. water	70	19.5 mm. water

Note: In every case described above there was inhibition of the spontaneous contractions which were in progress at the time of the stimulation. There was spontaneous recovery of tonus and contractions after cessation of the vagus stimulation.

In each case in experiments VIII and IX after recording the above observations the nerve was stimulated with "rapid induced shocks" of the same strength as the slow induced shocks. In each case a contraction followed stimulation of the nerve with this type of stimulus.

each case there was an inhibition of tone observed (see table 2). In these experiments after the inhibitory effect was obtained with a given strength of current using "slow induced shocks"—that is, at the rate of 30 to 40 per minute, the rate was increased to that of "rapid induced shocks" (2400 per minute). With the later stimulus, there was a latent period after which a brief period of inhibition was followed by a contraction. The procedure of stimulating the vagus with slow

induced shocks was used as a routine measure in twenty turtles in addition to those of this series proper. In these animals the vagus nerve was stimulated with slow induced shocks forty-seven different times. In thirty-three cases a distinct inhibition of gastric tone resulted from the stimulation. In fourteen cases there were no changes observed. It may be further noted from this group of experiments that stimulations were repeated on the same nerve as much as six times. Each stimulation resulted in inhibition of gastric tone which was followed by recovery. This recovery occurs rapidly. It is therefore evident that either gastric motor stimulating or gastric motor inhibitory effects may be obtained from the stomach of the turtle according to the rate of electrical stimulation of the vagus fibers. A slow rate of stimulation induces relaxation of tone and decreased contractility and a rapid rate is followed by a brief inhibition which precedes a powerful contraction. No evidence of a long-continued increase in gastric tone as the result of vagus stimulation was ever obtained.

Action of the cervical sympathetic nerve: After the effects obtained from stimulation of the cervical portion of the combined vago-sympathetic nerve, an attempt was made to determine whether or not the inhibitory affects described were due to stimulation of the sympathetic nerve fibers.

All the animals were prepared in the same manner as the preceding series.

Stimulation of the central end of the sectioned cervical sympathetic nerve (that is, the end connected with the spinal cord) in fourteen cases caused no effects. In five other cases after stimulation of the nerve, there was failure of gastric motility, but we have not succeeded in demonstrating in these cases that this failure was due to the sympathetic stimulation. In three animals, stimulation of the peripheral end of the sectioned cervical sympathetic nerve (that is, the end not connected with the spinal cord) caused no recorded changes in gastric tone.

Effects of section of both vagi: This series of experiments was performed to study the effects of section of both vagi on gastric tonus. All the turtles were prepared according to the method of Carlson and Luckhardt (5). The animals were decerebrated and given artificial respiration. The carapace was open in four experiments and closed in one. In those experiments in which the carapace was open, the stomach was dissected free from all of the surrounding membranes. Thus the effects recorded are those obtained only from the stomach

uncomplicated by possible mechanical changes due to muscle changes of the lungs.

The effects of section of both vagi are twofold. First, there may or may not be a powerful contraction of the stomach at the time of the section. This same effect has been noted by Doyon (24) and also by Contejean (16) and is probably the result of the mechanical stimulation of the motor fibers in the vagi by the section. On the other hand, the mere handling of the nerve in the fingers often causes a drop in tone and an inhibition of contractions. This occurs in turtles in very good condition. Doyon (3) noted that often simple ligature is sufficient to stop the movements of the stomach. Second, the intra-gastric pres-

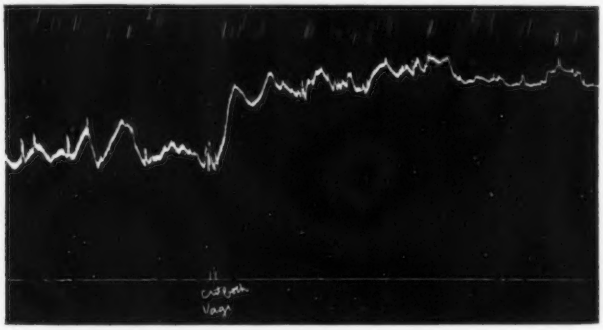

Fig. 4. Effects on intra-gastric pressure of section of both vagi. Turtle—Experiment V. May 1, 1920—Carapace open, artificial respiration, balloon in stomach, water manometer. Both vagi sectioned at point indicated on tracing.

sure is greater than it was before section of the vagi (see fig. 4). The accompanying table contains the details of the experiments.

The procedure of cutting both vagi in an attempt to gain an insight into the normal tonic action of the vagi has been tried by many observers. Among these is the work of Cannon (17) on cats during digestion; he noted that there was a temporary loss of tone and a slowing and weakening of the peristalsis, which in respect to rate was restored in a few days. On the other hand Carlson (18), working on dogs, noted that section of both vagi leaves the empty stomach on the whole hypo tonic permanently at least for a period up to 3 months after the operation. This work on mammals has been more or less confirmed by workers on frogs. Ravitsch (19) noted a paralysis of the stomach in

frogs after double vagotomy. Patterson (20) observed after section of the vagi in the frog that the contractions of the empty stomach were only slightly changed in rate and regularity. The stomach according to this observer is on the whole hypotonic. In a further study of double vagotomy in the frog, Patterson (21) noted an increase in the volume capacity of the stomach after section of both vagi. This is temporary, there being a recovery to normal after 8 to 10 days.

On the other hand Contejean (16) noted that in frogs whose stomach he had filled with salt solution by pouring it down the esophagus, after section of both vagi there was an increase in the movements of the stomach. This is in many respects confirmatory to the work of Goltz (11) who noted on curarized frogs which he had "completely pithed" that there were more "lively movements" of the stomach than in those

TABLE 3

Effects of section of both vagus nerves on intra-gastric pressure. Recorded in millimeters water. Carapace open = Two large holes in carapace, lungs exposed, stomach free from all surrounding tissues. Artificial respiration

DATE	EXPERIMENT	CONDITION OF CARAPACE	INTRA-GASTRIC PRESSURE BEFORE SECTION OF BOTH VAGI—MM. WATER	INTRA-GASTRIC PRESSURE AFTER SECTION OF BOTH VAGI—MM. WATER
April 15.....	I	Closed	25–27	39–40
April 16.....	II	Open	30	35
April 17.....	III	Open	31	40
April 20.....	IV	Open	15	32–35
May 1.....	V	Open	35	63

frogs which he had not pithed. He further noted that in those animals in which he had not injured the central nervous system the stomach was relaxed and there were no movements as long as the central nervous system was alive. "The stomach movements became very great when it died out."

It may be concluded that under these conditions, that is, in short time acute experiments with the animals decerebrated, the shell open and the stomach dissected free from all surrounding tissues, in winter turtles, there is an increase in intra-gastric pressure after section of both vagi. The turtles which were obtained during the summer did not show this increase in intra-gastric pressure after section of both vagi. There are several factors which may be involved in the difference in summer and winter experiments on this point. First, the reflex inhibitory mechanism is probably more active at the higher

body temperature of the summer and hence the traumatism necessary in preparing the animals for stomach work may lead to a greater depression of gastric motility than if the animals are in the sluggish winter condition. Second, there may be metabolic changes in the summer animals associated possibly with breeding season which alter the vagus responses.

Effects of injections of nicotine and atropine: A series of experiments was made to study the effects of stimulation of the vagus nerve on gastric tonus after injections of nicotine and atropine. The animals were prepared in the same manner as the preceding series. In addition the external jugular vein was cannulated for injection purposes. In all of these experiments the carapace was opened and the stomach freed from all the surrounding tissues. Rapid induced shocks at the rate of 2400 per minute were used for stimulation. In three cases pilocarpine was injected to increase the intra-gastric pressure.

The effects on gastric tonus of stimulation of the vagus nerve after nicotine may be divided into three groups, depending on the quantity of nicotine injected. First, $\frac{1}{8}$ to $\frac{1}{4}$ mgm. In five cases injection of this quantity of nicotine was followed by a drop in intra-gastric pressure. In two of these cases there was an inhibition of the spontaneous contractions in progress. In one case this decrease in intra-gastric pressure associated with an inhibition of contractions was obtained following an injection of pilocarpine. Under these conditions stimulation of the vagus nerve with rapid induced shocks at the rate of 2400 per minute has the usual effect, that is, a brief inhibition followed by a powerful contraction. Second, on increasing the quantity of nicotine injected a stage may be reached in which stimulation of the vagus nerve had no recorded effects on the stomach. The amount of nicotine required to produce this condition varies in different animals. These results are in accord with those obtained by Doyon (15) who noted that after small doses of nicotine, vagus action was left intact. He further stated that after larger doses vagus action is lost, and he obtained no changes in the gizzard of the pigeon on stimulation of the vagus nerve. Third, stimulation of the vagus nerve with rapid induced shocks after a still larger quantity of nicotine is followed by a decrease in intra-gastric pressure. The quantity of nicotine required is variable in different animals and in this series ranged from 2.0 mgm. to 10.5 mgm. Therefore if a sufficient quantity of nicotine be injected, under the conditions of the experiments, stimulation of the vagus nerve with rapid induced shocks is followed only by a decrease in intra-gastric

pressure. This inhibitory effect after nicotine was observed in seven experiments in this study. This observation does not agree with the work of Doyon (15) on the pigeon's gizzard. This observer states that at no time did the vagus seem to possess an inhibitory influence on the gizzard of the pigeon after nicotine.

Three experiments were carried out to study the effects on intra-gastric pressure of stimulation of the vagus after atropinization. One milligram of atropine was injected in each case. Atropine causes a relaxation of the stomach tonus and cessation of the spontaneous contractions. Stimulation of the vagus with induced shocks at the rate of 2400 per minute is followed by a further drop in intra-gastric pressure from which there is prompt recovery after the stimulation has ceased.

These results confirm those of other observers. Langley (10) on the dog noted that when atropine was injected the inhibitory influence of the vagi came more and more to the front. After a certain quantity of the drug, which he did not determine, vagus stimulation with all strengths of current caused only a fall in gastric tone. This same has been noted by Meltzer and Auer (22), Smith (12) and Batteli (23).

From this series of experiments we conclude that in the turtle the inhibitory action of the vagus nerves is more resistant to atropine than are the motor effects.

CONCLUSIONS

1. Stimulation of the peripheral and of the sectioned vago-sympathetic nerve in the turtle with "rapid induced shocks" (2400 per minute) after a latent period of varying lengths leads to a brief inhibition of gastric tonus which is followed by gastric contraction. This confirms the work of Hopf (7) on the frog.

2. Repeated short periods of stimulation of the vago-sympathetic nerve with rapid induced shocks at the rate of 2400 per minute is followed by:

a. Progressive lengthening of the latent period of gastric motor responses.

b. Progressive decrease in the amplitude of the gastric contractions to a stage where no contraction follows stimulation of the nerve.

c. When this condition of failure of the stomach to respond to stimulation of one vagus has been reached, stimulation of the other vagus may or may not induce a contraction. If a contraction follows stimulation of this second vagus, a more rapid failure to respond to repeated

stimulation of this nerve ensues than if the other nerve had not been stimulated.

3. Stimulation of the peripheral end of the vagus nerve with induced shocks at the rate of 30 to 40 per minute causes relaxation of gastric tone with or without inhibition of the spontaneous contractions. This type of stimulation repeated at short intervals does not lead to fatigue or failure of the inhibitory response such as follows repeated stimulation with rapid induced shocks (2400 per minute).

4. Whether stimulation or inhibition of gastric motility will follow electrical stimulation of the vagus nerve depends on the rate of stimulation and not the intensity of the current.

5. Stimulation of the central end of the sectioned cervical sympathetic nerve and also the peripheral end of the same nerve usually has no recorded effect. At times stimulation of the central end of the cervical sympathetic nerve seems to be followed by an inhibition which is long-delayed and lasting in its effects, but the evidence on this point is not conclusive.

6. Section of both vagi in acute experiments in the decerebrate turtle in which the carapace has been opened and the lungs and stomach freed from all surrounding tissues is followed by a rise in intragastric pressure. This rise continues throughout the remainder of the experiment.

7. Small doses of nicotine cause an inhibition of the automatic contractions and of the tone of the stomach. Recovery from this inhibition is prompt.

8. If the vagus nerve is stimulated with induced shocks at the rate of 2400 per minute after a sufficient quantity of nicotine has been injected intravenously, only inhibitory effects on gastric motility and tonus are obtained.

9. Stimulation of the vagus nerve after the injection of atropine is followed by a drop in intra-gastric pressure. This confirms the work of other observers.

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THE VITAMINE CONTENT OF HONEY AND HONEY COMB[1]

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The object of the experiments here reported was to determine whether certain typical strained and comb honeys contained the growth-promoting vitamines fat-soluble A, water-soluble B, and antiscorbutic vitamine, and if so, to what degree.

We are not aware of any experiments which have been carried out to determine whether or not either strained honey or comb honey might contain the fat-soluble vitamine. Dutcher, in 1918 (1), found that a specimen of basswood and white clover honey examined by him contained a small amount of antineuritic vitamine which, however, he considered negligible. There was very little evidence of the presence of the antineuritic substance in dilute unevaporated nectar, although corn pollen was found to be relatively rich in it. Bachmann (2), using the growth and multiplication of yeast cells as an index, arrived at somewhat similar conclusions. The identity of the antineuritic vitamine studied by Dutcher with the growth-promoting vitamine called water-soluble B is not, however, conceded by Emmett and Luros (3). The use of yeast cells as indices of the presence and amount of this vitamine has also been adversely criticised by Souza and McCollum (4). Studies of the water-soluble B content of honey from the standpoint of animal growth promotion have not previously been reported.

The antiscorbutic potency of honey has been studied by Faber (5) in a paper reported after the present work was completed. This author used white sage honey extracted from the comb on the day of administration and did not find it to contain antiscorbutic vitamine in protective amounts.

Water-soluble vitamine in honey. White rats of suitable age and weight were obtained from the Rat Colony of The Wistar Institute of

[1] The expenses of this investigation were defrayed in part from funds furnished by Mrs. M. H. Henderson.

Anatomy, Philadelphia. They were divided into three groups, rats of each litter being distributed as uniformly as possible throughout the different groups so as to make the comparisons as direct as possible.

The diets used were as follows:

Group I, 9 rats

500 grams casein (purified)
1575 grams starch
375 grams butter (purified)
50 grams inorganic salt mixture

This diet was known from previous experience to contain all essential nutrients, except the vitamine water-soluble B, in which it was deficient.

Group Ia, 5 rats

212 grams casein
394 grams starch
492 grams blended honey
188 grams butter
25 grams inorganic salt mixture

This diet was known to contain no water-soluble B vitamine, except such as might be found in the blended honey.

Group Ib, 5 rats

Diet same as for group Ia, except that 492 grams honey were substituted by 492 grams of white clover honey. This diet was known to contain no water-soluble vitamine, except such as might be present in the white clover honey.

The casein used in these experiments was purified by several reprecipitations from alkaline solution with acetic acid and thoroughly washed. The butter fat was obtained by siphoning off the melted fat of unsalted butter from the sediment of curd and water.

The salt mixture was prepared from chemically pure salts and had the same composition as that previously used by us (6).

In the diets of groups Ia and Ib, 50 per cent of the starch was replaced by an isodynamic equivalent of honey.

The rats were kept in sanitary cages and were permitted to eat as much as they desired of the diet of their particular group, together with an abundance of water. They were weighed once each week.

After four weeks the diets were changed so that the rats of group I, which had received no honey, were now subdivided into two groups, half of which received blended and the other half white clover honey. The rats of groups Ia and Ib, which had been receiving honey, now had this replaced by starch.

After another period of two weeks a second change of diet was made, which consisted in replacing all these previous food mixtures by milk which was known to be adequate for growth and to contain water-soluble B.

The results are given in charts 1 and 2, which show the losses and gains in weight for each rat. Curves showing the average gains and

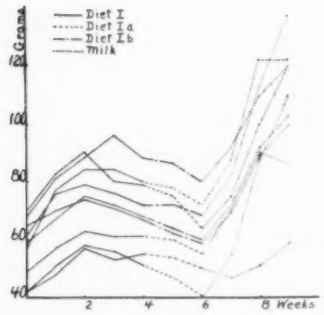

Chart 1

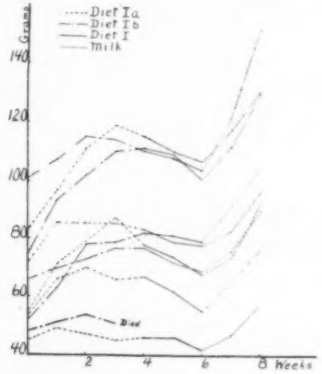

Chart 2

Water-soluble vitamine in honey

losses for each group are also charted in the final figure which summarizes the results (chart 5).

An examination of the data will show that none of the rats on either of these three diets was able to thrive and grow in a normal manner. Apparently, therefore, all three diets were deficient in the essential vitamine, water-soluble B.

It appeared, therefore, that neither blended nor white clover honey contained sufficient water-soluble B to permit normal growth when they were present in the diet or to permit the resumption of growth when they were added to a diet containing none of this vitamine.

This did not necessarily indicate that the honeys studied contained none of this vitamine. If we look at the average curves in the final

figure, we see that at the beginning there was an average difference in weight of 4 grams between the "starch" rats and the "blended" rats, and that at the end of five weeks the difference had increased to 9 grams. In the same way the difference in favor of white clover honey increased from 11 to 17 grams.

The differences were not great enough to be conclusive but may most reasonably be considered to indicate the slightly greater efficiency of honey for growth, due to the presence of small amounts of the water-soluble vitamine.

Fat-soluble A vitamine in honey. Three groups of rats were used, being selected as in the preceding experiment. The following diets were used:

Group II, 10 rats

360 grams casein
1134 grams starch
270 grams Crisco
36 grams salts
90 grams yeast

This diet was known to be adequate, except for the absence of the essential vitamine, fat-soluble A.

Group IIa, 5 rats

320 grams casein
504 grams starch
630 grams blended honey
241 grams Crisco
81 grams yeast
32 grams salt

This diet was known to contain no fat-soluble A vitamine, except such as might be present in the blended honey.

Group IIb

Diet same as for group IIa, except that 630 grams of blended honey were substituted by 630 grams of white clover honey. This diet was known to contain no fat-soluble vitamine, except such as might be present in white clover honey.

In diets IIa and IIb 50 per cent of the starch of diet II was replaced by isodynamic quantities of the honeys studied.

The data with regard to the rats of these three groups are given in charts 3 and 4 and the summarizing chart (chart 5).

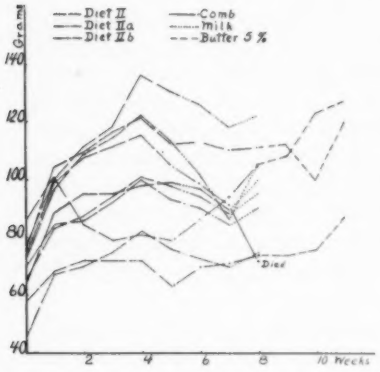

Chart 3

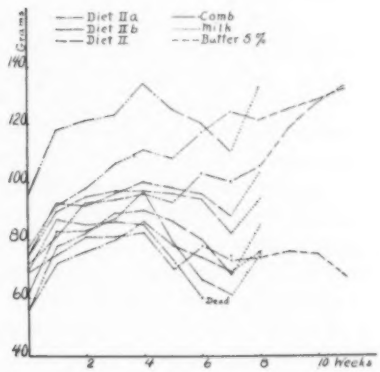

Chart 4

Fat-soluble vitamine in honey

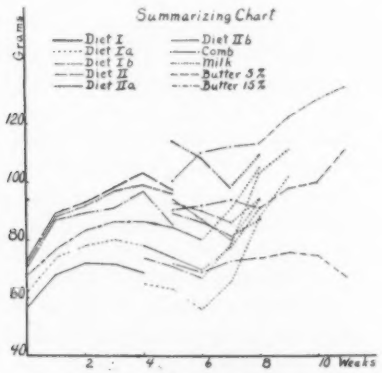

Chart 5. Vitamines in honey

It will be noted that after a period of five weeks on the above-mentioned diets a change was made so that of the rats not previously receiving honey, half were now given blended and half white clover honey, while the rats previously receiving these two honeys had them substituted by starch for a period of two weeks.

In certain cases comb honey was given to determine the vitamine content of this product, and later butter was added for comparison, as this fat was known to contain an abundance of fat-soluble A.

The rats not receiving comb honey were finally given milk (an adequate food) as a control, to show that failure to grow had been solely due to a dietary deficiency and not to disease or other accidental causes.

From the data on these three groups of rats it will be noted that on neither diet were the rats able to grow and thrive in a normal manner. Apparently, therefore, all three diets were deficient in the essential vitamine, fat-soluble A. The blended and white clover honeys used could not, therefore, have contained sufficient fat-soluble A to permit of normal growth when they were present in the diet, or to permit the resumption of growth when they were added as the sole source of this vitamine to a diet entirely lacking in this essential element.

That normal growth was not attained with honey as the sole source of fat-soluble vitamine in the diet did not necessarily indicate that the honey contained none of this vitamine. If we look at the data on the various groups of rats, we see that animals of litter 5 gained a little more in weight on white clover honey than animals of the same litter receiving no honey. Litters 2 and 3 showed, however, a tendency in the opposite direction, so that the average curve for rats of all litters is quite similar for the diets with and without blended or white clover honey, so that if this vitamine be present in strained honey it must be in small amount.

That a minimal amount of fat-soluble A may be present in honey would be indicated further by the fact that our data show it to be present in comb honey.

The eight animals fed for five weeks on a diet lacking fat-soluble A all showed signs of dietary deficiency; one, in fact, was beyond recovery.

When comb honey was added to their diet, all but this one showed cessation of decline and distinct gains in weight. Comb honey caused a resumption of growth in these animals, indicating the presence of distinct amounts of fat-soluble A.

For comparison the comb honey diet was followed by one containing butter in amounts of 5 to 15 per cent. Butter is known to be rich in fat-soluble vitamine. Hence the fact that the animals grew about as well on comb honey as when 5 per cent of butter was added indicates that comb honey contains a moderate amount of fat-soluble vitamine.

Antiscorbutic vitamine in honey. Guinea pigs develop scurvy when deprived of foods containing the antiscorbutic vitamine. To deter-

mine whether this vitamine was present in honey, guinea pigs were divided into three groups as follows:

Group III, 9 guinea pigs

1000 grams oats
100 grams dry milk
262 grams starch
50 grams yeast
50 grams butter
Ad lib. hay (heated to destroy antiscorbutic vitamine).

This diet was known to contain all essential nutrients with the exception of the antiscorbutic vitamine. Hence it should give rise to scurvy.

Group IIIa, 6 guinea pigs

Diet same as that of group III, except 262 grams starch replaced by 300 grams of blended honey. This diet contained no antiscorbutic vitamine, except such as might be present in the blended honey. The honey was mixed with the oats.

Group IIIb, 6 guinea pigs

Diet same as that of group IIIa, except 300 grams of blended honey replaced by 300 grams white clover honey. This diet contained no antiscorbutic vitamine, except such as might be present in the white clover honey.

The guinea pigs were kept in sanitary cages, were weighed at weekly intervals and observed for the development of the first signs of scurvy.

The table shows the weights of the guinea pigs and the dates upon which symptoms of scurvy were noted in each case. Scurvy was diagnosed by the external symptoms, by post-mortem examinations showing bloody extravasations, and by histological examination of the bones.

The following are typical microscopical findings on post-mortem examination of guinea pigs fed on diets with and without honey:

Microscopic study of bones: 1. Honey-fed pig. Femur: The muscles about the joint showed diffuse and focal hemorrhages both between and replacing muscle fibers, many of which were fragmented and manifested nuclear degeneration. In addition there was an inflammatory exudate of mononuclear and a few polynuclear leukocytes. The endothelium lining the blood vessels was swollen.

The line of junction between bone and costal cartilage was irregular, with proliferating trabeculae of cartilage cells extending into the marrow. There was a slight degree of necrosis of the marrow surface of the costal

Experiments on guinea-pigs

DIET III: Oats, 1000
Dry milk, 100
Starch, 262
Yeast, 50
Butter, 50

DATE	PIG NUMBER								
	1	2	3	4	5	6	7	8	9
1-23-20	235	243	179	273	320	218	246	238	225
1-30-20	270	295	196	328	385	266	315	258	282
	S 2-6	S 2-2	S 2-1	S 2-3	S 2-3	S 2-6	S 2-2	-	S 2-6

DIET IIIa: 300 grams blended honey in place of starch

DATE	PIG NUMBER					
	10	11	12	13	14	15
1-23-20	317	412	261	228	261	274
1-30-20	378	475	296	257	326	321
	S 2-5	S 2-5	S 2-1	S 1-30	S 2-1	S 2-5

DIET IIIb: 300 grams white clover in place of starch

DATE	PIG NUMBER					
	16	17	18	19	20	21
1-23-20	389	267	279	289	336	260
1-30-20	431	336	323	351	406	315
	S 2-5	S 2-5	S 2-1	S 2-5	S 2-3	S 2-1

Pig 3—$CHCl_3$ 2-6-20 Gross signs of scurvy. Ribs and femurs examined histologically.

Pig 14—$CHCl_3$ 2-6-20 Gross signs of scurvy; thighs especially. Femurs. Discontinued 2-6-20.

"S" indicates date first signs of scurvy appeared.

cartilage. There were many minute hemorrhages in the marrow substance and irregular masses of cartilage and bone especially marked beneath the periosteum. The inner surface of the epiphyseal cartilage is irregular but shows no evidence of necrosis.

2. Control pig. Femurs and costo-chondral junctions of ribs showed changes cited above. No muscle changes noted.

The changes described correspond to descriptions of bone lesions of experimental scurvy in guinea pigs by Jackson and Moore (7).

An examination of the data shows that all of the guinea pigs (with one possible exception) on all three diets with and without honey developed typical scurvy within two weeks. It is apparent, therefore, that neither blended nor white clover honey was able to protect against the development of scurvy and hence could not have contained appreciable amounts of the antiscorbutic vitamine.

The response of the human stomach to honey. Experiments were carried out on a normal man, to determine the influence of honey upon gastric digestion as part of a general study in this laboratory of the response of the human stomach to foods. The subject was first given 40 grams of whole wheat bread alone. The contents of the stomach were analyzed for acid and pepsin at 15-minute intervals. The experiment was then repeated, adding to the bread 20 grams blended honey. The results are plotted in figure 6, the upper curve representing total acidity; the next lower, free hydrochloric acid; the third, pepsin, and the fourth, amino-acid nitrogen.

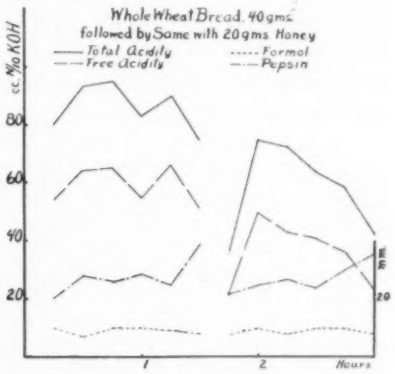

Chart 6. Gastric response to honey

An examination of the chart will show that the bread with honey was digested and left the stomach as quickly as the bread alone. Similar pepsin values were obtained; and while there was a slight depression of acidity, such as always follows the ingestion of foods containing much sugar (8), digestion was completed as soon as with bread alone, although the addition of the honey had practically doubled the food value of the product from the energy standpoint.

The use of honey with bread and in similar ways would, therefore, appear to be generally preferable in the case of children to the eating of candies. Honey serves to make the highly nutritious bread more palatable, leading to a greater consumption of body-building foods

instead of depressing the appetite as is likely to be the case with candies which are eaten between meals. At the same time honey furnishes to the body very considerable amounts of energy in the most available form. The high place given to it in the diet is therefore well deserved.

CONCLUSIONS

Strained honey was found, by experiments on albino rats, not to contain more than minimal amounts of the growth-promoting accessory substances, fat-soluble A and water-soluble B. Comb honey was found to contain the fat-soluble vitamine in moderate amounts. Honey did not protect guinea pigs against the development of scurvy. The addition of honey to bread did not appreciably delay its digestion in the human stomach.

The authors desire to express their thanks to Dr. Edward Weiss, who performed autopsies on the guinea pigs used in these experiments, for the pathological report included in this paper.

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SUGAR IN THE BLOOD OF THE DOG-FISH AND OF THE SAND SHARK

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The common dog-fish, Mustelis canis, has been used to some extent for physiological experimentation and it seems to me that this use should be extended. It was with this thought in mind that the following observations were made. The work was done in the Marine Biological Laboratory, Woods Hole, Massachusetts, during the months of June, July and August, 1915. I wish at this time to express my appreciation for the opportunity afforded me by the Laboratory management.

Claude Bernard (1) was probably the first to determine the sugar in the blood of an elasmobranch. In 1874 he reported the blood sugar of a shark to be 51 mgm. per 100 cc. of blood. After this little, if anything, seems to have been done until Diamare (2) undertook the study of the sugar metabolism in elasmobranchs early in the present century. He, with Montuori (3), reported blood sugar values for several members of this group, among which are two genera of dog-fish, Scyllium and Mustelus. In none of the forms studied did they find sugar normally present in the blood. Later Diamare succeeded in demonstrating the presence of sugar in the blood of these fish after removal of the pancreas and after glucose had been injected. Peculiarly enough he seldom found glycosuria even after he had removed the pancreas. This peculiarity he attributed, apparently however without any direct experimental evidence, to a relatively low permeability of the kidney for sugar.

In 1914 Mlle. Fandard and Rane (4) reported blood sugar values for several species of fish, among which was one individual of Mustelus vulgarus. This one specimen gave a blood sugar value of 78 mgm. of sugar per 100 cc. of blood. They reported no difficulty in demonstrating sugar in the blood of any of the elasmobranchs and suggested that possibly the difficulty experienced by Diamare was due to the fact that

the method chosen for analysis was not appropriate for use with elasmobranch blood with its high content of urea. This, however, is not necessarily a criticism of his studies of the urine after removal of the pancreas, and if the absence of sugar in the urine of these fish under such circumstances should be confirmed, a careful study of the sugar metabolism of this group should prove of great value.

In the present work the fish, after capture, were kept in traps which were exposed to tide water, until required for the actual experiment in the laboratory. During the experiment they were kept in a tank which had a surface of about two square meters and a depth of about twenty centimeters. Frequent oxygen determinations were made upon the water of the tank during the latter two-thirds of the experiments.

In order to obtain the blood the fish were removed from the tank and as rapidly as possible the caudal artery was severed and the blood was caught in a tared beaker as it flowed from the severed artery. The beaker contained about 10 cc. of a 1 per cent solution of potassium oxalate to prevent coagulation. Two methods were used for the removal of the protein from the blood: 1st, the phosphotungstic acid method described by myself (5); and 2nd, the dialysed iron method of Rona and Michaelis (6) as modified by Macleod (7). In either case the reducing power of the blood was determined by the "unified method" of Munson and Walker (8), and calculated as glucose.

Though ordinarily the presence of reducing sugar was easily demonstrated, occasionally it seemed to be absent or to be present only in very small quantities. This condition appeared to be abnormal, however, rather than the normal one as maintained by Diamare. The cases showing no sugar or an unusually small amount are collected in table 1.

A study of this table will show that of the eight individuals showing a low blood sugar, six were patently subnormal or had been exposed to very abnormal conditions just before the blood sample was obtained.

Fandard and Ranc (4) speak of the ease with which asphyxial hyperglycemia may be brought about in fishes and further state that fish are more susceptible to this type of hyperglycemia than are mammals. Their work, at least so far as published, does not seem to justify so categorical a statement and for this reason it was thought desirable to study this point in some detail.

In carrying out this study the fish were kept in the tank already described in which the sea water was maintained at an oxygen concentration as nearly normal as was possible. Oxygen determinations, as

already noted, were not made in the earlier experiments but the conditions as to rate of water flow and number of fish kept in the tank were the same as in the later experiments so that the oxygen content of the water would presumably be the same as in those experiments in which it was determined.

In this series the fish were removed from the water and allowed to lie in the air for varying periods of time before the blood sample was taken. The results are shown in table 2, and summarized in figure 1.

From the table and more strikingly from the figure it is seen that there appears to be a distinct and very rapid rise in the blood sugar during the first two minutes of asphyxia, which is followed by a much slower fall until a level, very near the value which may be assumed to be normal, is reached about six minutes after the asphyxia was begun.

TABLE 1

To illustrate that an unduly small amount of sugar in the blood of dog-fish is usually accompanied by other evident subnormal conditions

EXPERIMENT NUMBER	DATE 1915	SUGAR IN 100 GRAMS OF BLOOD	REMARKS
		mgm.	
5	July 2	None	Fish not vigorous
24	July 23	29	Oxygen in water only 0.7 cc. per liter
25	July 24	41	Oxygen in water only 1.9 cc. per liter
26	July 26	32	Out of water for 15 minutes
27	July 26	Trace	Apparently normal
28	July 27	None	Out of water 5 minutes. Nonreactive, limp
30	July 28	Trace	Out of water 8.5 minutes
37	August 3	Trace	Apparently normal

In the absence of an explanation, this may, for the time being, be presumed to result in some way from the weakened condition of the fish and so asphyxia is to be associated with certain other conditions which result in inactivity and are correlated with a low concentration of sugar in the blood. Until more is known of the mechanism of the phenomenon one is not warranted in making a statement as to which is cause and which is effect.

It may be of interest to note that the average blood sugar for the entire series of dog-fish is 65 mgm. per 100 gm. of blood, which is of the same order of magnitude as we have found for the cat and dog, i.e., 69 mgm.

While working upon the dog-fish opportunity offered itself for the determination of the sugar in the blood of four sand sharks, Carcharias

littoralis. In the case of the first three the blood was obtained from the vertebral artery by means of a dorsal incision in the neck. In the last instance it was obtained, as in the dog-fish, by section of the caudal

TABLE 2

To show the relation of asphyxia, produced by keeping dog-fish in air for varying periods of time, to the blood sugar

EXPERIMENT NUMBER	TIME IN AIR		OXYGEN IN 100 CC. WATER		SUGAR IN 100 GRAMS OF BLOOD	REMARKS
	Minutes	Seconds	Initial	Final		
			cc.	*cc.*	*mgm.*	
29	0	55	5.5	5.2	81	
44	1	15	5.2	5.2	66	
45	1	27	5.0	5.5	137	
27	1	30	5.2	5.2	Trace	See table 1
32	1	30	5.1	4.7	50	
31	1	50	4.9	4.4	86	
33	2	0	5.1	5.1	195	
36	2	10	4.8	5.0	87	
18	2	30			184	
20	3	15	5.0	4.6	67	
8	4	0			61	
10	4	0			232	
13	4	0			249	Highest sugar observed
19	4	25	4.6	4.9	182	
12	4	30			111	
21	4	35			101	
3	5	0			94	
9	5	0			101	
11	5	0			58	
28	5	0	5.2	5.1	Absent	See table 1
35	5	20			72	
6	6	0			104	
7	6	0			82	
17	6	0			51	
30	8	30	4.9		Trace	See table 1
16	9	30			82	
4	10	0			172	
26	15	0			32	See table 1

artery. This fish had been kept in a small tank for the twenty-four hours previous to the taking of the sample and had been subjected to another experiment. As a result it was in a very poor condition. The results are given in table 3.

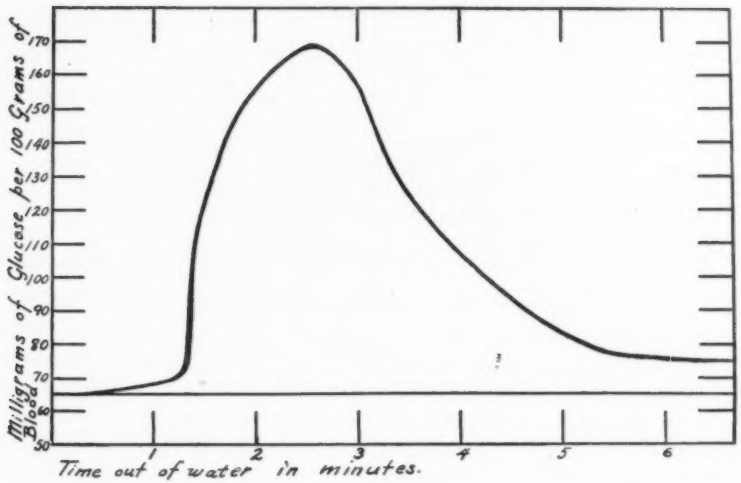

Fig. 1

TABLE 3

The concentration of sugar in the blood of the sand shark

EXPERIMENT NUMBER	DATE 1915	SUGAR IN 100 GRAMS OF BLOOD	REMARKS
		mgm.	
1	July 13	77	Protein removed by dialysed iron
2	July 13	50	Protein removed by phosphotungstic acid
3	July 31	57	Protein removed by dialysed iron
4	August 24	27	Protein removed by dialysed iron

SUMMARY

1. Sugar is occasionally absent from the blood of Mustelus canis. This absence of sugar from the blood seems to be usually accompanied by a subnormal condition of the fish.

2. Asphyxia induced by exposure of the fish to air causes a rapid rise of the blood sugar which is followed by a fall to a value near normal.

3. The average concentration of sugar in the blood of dog-fish of the series is of the same order of magnitude as that found for the dog and the cat.

4. The concentration of sugar in the blood of sand sharks appears to be about the same as that in the blood of dog-fish.

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SUGAR IN THE BLOOD OF THE COMMON FROG

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The frog is so extensively used in the laboratory that it seemed desirable to us to have more data than are now available concerning the concentration and behavior of the sugar in its blood.

Comparatively little has been published upon the blood sugar of the frog and, so far as we have been able to find, nothing from America. The greater part of what has been published has been worked out upon very large frogs or even upon composite samples of blood taken from a considerable number of animals. This has been necessary in the past because of the large amounts of blood required for making the determinations. A number of micromethods are now available which have removed this difficulty.

The blood of frogs has been reported to contain concentrations of sugar varying from zero to 81 cgm. per 100 cc. of blood according to the author and the method used. Such a range of variation is very unusual if not unknown in other animals and indicates the necessity of a considerable number of determinations so that the true condition may be known.

Bang (1), using his own micromethod, determined the sugar content in the heart blood of normal winter frogs which had been kept in a cellar; he found it to vary between 0.02 and 0.03 per cent. After keeping the frogs for a few days at room temperature their blood sugar rose to a concentration of 0.04 to 0.05 per cent. Freshly captured Rana temporaria showed a blood sugar concentration in the month of May of 0.04 per cent and in the month of August of 0.04 to 0.05 per cent. About the same figures were obtained with Rana esculenta. Thus the blood sugar figures obtained by Bang range from 0.02 to 0.05 per cent.

Lesser (2) at first reported that normal frogs had no sugar whatever in their blood. He used the Rona-Michaelis dialysed iron method for removing the proteins, and was unable to detect any reduction of copper in the filtrate (Bertrand's method). He used for analysis a compos-

ite sample of blood weighing 15 to 20 grams which he obtained from several frogs. Although he found no sugar in the heart blood of normal frogs, he discovered that after the frogs had been subjected to "anoxybiosis" for 2 to 3 hours and then allowed to recover for 20 hours, a majority of those which survived showed a blood sugar content of 0.07 per cent (of three groups of frogs two showed 0.07 per cent and one none at all). The author does not describe the conditions by which the "anoxybiosis" was brought about.

The same author a few months later was able to show the presence of sugar in the blood of normal frogs. This time he used Bang's method. In the month of July, collecting the blood from the hearts of 16 to 30 frogs, he found in three successive samples 0.035, 0.04 and 0.036 per cent of sugar. Still later in a still more comprehensive investigation he found only 0.029 per cent.

Loewit (3) used large frogs (100 to 200 grams), from Hungary and Vienna. He drew the blood directly from the heart by means of a pipette. In normal winter frogs he found an average blood sugar content of 0.54 per cent, and in spring frogs of 0.81 per cent. Bang says that these values are improbable, being ten to forty times greater than those which he obtained himself. Loewit, however, used Bang's method. They seem to us in the light of the present work to be much too high.

Loewit was able to induce a condition of hyperglycemia and even of glycosuria by keeping the frogs on ice. This was especially noticeable with frogs obtained from Hungary. His "Kaltefrösche" showed a blood sugar content of 0.558 to 1.17 per cent.

Recently Brinkman and Mlle. Van Dam (4) have determined the blood sugar in frogs by Bang's micromethod. They found it to range from 0.040 to 0.065 per cent. They found no sugar in normal corpuscles.

All of the above determinations were made on the heart blood obtained either from several frogs or from individual frogs of very large size. Frogs commonly used in the laboratory are, unfortunately, small and it was therefore decided to use the ordinary frogs which are regularly supplied by dealers, Rana pipiens, as being more representative of the common laboratory animal. The frogs were obtained from Indiana, Wisconsin and Pennsylvania. A number of the frogs were kept in a refrigerator for two to nine days, and their blood sugar was then determined. Since the work was done in February, March and April, any change of the sugar content of the blood which might be due to the onset of the breeding season could be noted. Differences of blood sugar

figures which might be correlated with sex or weight would also be brought to light.

Method. The frogs were so small that it was impracticable to draw the blood from the heart by means of a pipette. Therefore it was found best to obtain the blood by cutting the heart open. To prevent contamination by the admixture of lymph or of peritoneal fluid the following procedure was employed. The frog was pithed or etherized, tied to a frog-board and weighed. A piece of skin, somewhat less than a centimeter square, was removed from the ventral surface of the body, directly over the heart. A portion of the sternum was removed so that the junction of the ventricle with the bulbus arteriosis was exposed. The opening in the body wall was too small to expose the whole heart. The pericardium was slit open, curved forceps were introduced between it and the ventricle, and the heart was gently drawn out. By this means the opening in the body wall was completely blocked by the heart. After thoroughly drying the heart and surrounding tissues with absorbent cotton the frog-board was inverted and held for a few seconds with the heart suspended. The purpose of this was to retard the flow of blood from the heart. The junction of the ventricle and the bulbus was now snipped with fine scissors and the blood which flowed from it was caught in a small watch glass on which there were sprinkled a few crystals of potassium oxalate.

This procedure is satisfactory even for frogs as small as 20 grams. The heart should continue to beat for some time after the sample is taken, but as a rule the frogs were killed at once, their body cavity opened and the viscera examined.

After collecting the blood, two or three samples of 0.2 cc. each were measured out and added to the sodium sulphate solution as required in the blood sugar method of MacLean (5), which was used in all of the determinations. This micromethod consists of a simple procedure involving no unusual precautions except the avoidance of evaporation during the removal of the proteins. The N/400 sodium thiosulphate solution was freshly prepared from a N/10 stock solution and the alkaline copper solution was standardized each time determinations were made.

Results and discussion. Series I consisted of 26 normal male and female frogs which varied in weight from 21 to 63 grams. The frogs were kept in a vivarium at a temperature of about 18°C. The detailed results are given in table 1, and the results are summarized in table 2.

A study of these tables will show first that there is no significant variation of the blood sugar with the onset of spring when the series is considered as a whole or when the males alone are considered. This may be contrary to what one would expect, though as a matter of fact

TABLE 1

The weight, sex and blood-sugar of the normal frogs studied

EXPERIMENT NUMBER	DATE 1920	SEX	WEIGHT	GLUCOSE IN 100 CC. OF BLOOD	REMARKS
			grams	*mgm.*	
	February				
1	25	F	52	11	
2	25	F	53	34	
3	27	F	43	50	
4	27	M	35	26	
	March				
5	3	F	63	50	
6	3	F	61	52	
8	10	F	32	24	
9	12	F	25	40	
10	12	F	21	39	
11	12	M	46	33	
12	17	M	40	24	
13	17	M	31	36	
14	19	F	29	42	Ether; eggs well developed
15	19	M	28	40	Ether
16	24	M	41	30	
	April				
23	2	F	34	43	Eggs well developed
24	5	F	63	53	Eggs well developed
26	5	M	23	38	
27	7	F	33	46	
29	7	M	26	38	
31	9	F	29	38	
33	14	M	37	33	Ether
34	16	F	29	38	Ether
38	21	M	26	41	
41	30	M	21	38	
42	30	M	31	28	

it is not out of harmony with the results reported by Bang. It must be borne in mind that our winter results were not obtained upon hibernating frogs but upon those kept at a temperature of 18°C. and that such frogs are fairly active, even in winter.

It will be noted further that there seems to be no relation between the weight of the animal and the amount of sugar in its blood (lines 1, 2 and 3 of table 2).

A comparison of the sexes reveals an entirely different condition of affairs. Lines 6 and 7, table 2, show strikingly that the blood of the females contains distinctly more sugar than does that of the males and further, lines 8 and 9 of the same table indicate that this condition is directly related to the condition of the eggs. This is of considerable interest when related with the work of Laurence and Riddle (6) upon sexual differences in the phosphorus and fat in the blood of fowls.

TABLE 2

Summary of the results detailed in tables 1 and 4

	NUMBER OF FROGS	WEIGHT	GLUCOSE IN 100 CC. OF BLOOD
		grams	*mgm.*
Average for the entire series	26	35	37
Average for those lighter than 35 gm	16	28	37
Average for those heavier than 35 gm	10	50	37
Average for those with less than 37 mgm. sugar	11	38	28
Average for those with more than 39 mgm. sugar	15	34	43
Average for all males of this series	12	32	33
Average for all females of this series	14	41	40
Average for all females with poorly developed eggs	11	40	38
Average for all females with well developed eggs	3	42	46
Average for all frogs kept in icebox	12	34	48
Average for all male frogs kept in ice box	7	29	47
Average for all female frogs kept in ice box	5	40	50

In the case of mammals where ovulation is not so great a factor such differences are not so apparent (cf. Scott, 7).

Four frogs were used which, upon autopsy, were found to be abnormal. The results for these animals are given in table 3. From this table it will be seen that in two of the three in which the liver was abnormal no sugar at all was recovered, while the third which was apparently in the same condition gave the usual amount of sugar. The most obvious conclusion is that, in the two cases, the liver had lost its glycogenolytic function. While this is the most obvious explanation, it may of course not be the correct one and in the meantime it is interesting to compare these frogs with the abnormal dog-fish reported by Scott, which gave little or no sugar in their blood.

Blood sugar in refrigerated frogs. Twelve frogs were used, seven males and five females. They were kept from two to nine days at a temperature of 8° to 9°C. The average weight of these frogs was 34 grams. The average blood sugar found in the males was 47 and in the females 50 mgm. per 100 cc. of blood. The average for the entire series 48 mgm. per 100 cc. It will be observed that all of these figures

TABLE 3

Weight, sex and blood sugar of four abnormal frogs

EXPERIMENT NUMBER	DATE, 1920	SEX	WEIGHT	GLUCOSE IN 100 CC. OF BLOOD	REMARKS
			grams	*mgm.*	
7	March 10	M	28	Absent	Suppurating liver
25	April 5	M	33	Absent	Suppurating liver
30	April 9	M	23	31	Blood appeared to be watery
39	April 23	M	25	39	Suppurating liver

TABLE 4

Weight, sex and blood sugar of frogs kept in an ice box at 8°C.

EXPERIMENT NUMBER	DATE, 1926	SEX	DAYS IN COLD	WEIGHT	GLUCOSE IN 100 CC. OF BLOOD	REMARKS
				grams	*mgm.*	
17a	March 26	F	2	57	53	Eggs well developed
18	March 26	F	2	38	52	Ether
19	March 31	M	7	33	74	
20	March 31	M	5	41	52	
21	April 2	F	2	40	51	
22	April 2	F	2	35	47	Eggs well developed
28	April 7	M	2	25	26	
32	April 14	F	7	30	49	Eggs well developed
35	April 16	M	2	28	38	
36	April 16	M	7	22	44	
37	April 21	M	2	24	47	
40	April 23	M	9	32	46	

are higher than those obtained from frogs kept at ordinary room temperature. This is of course a qualitative confirmation of Loewit's results, though we were able to get no such figures as he reported. It is interesting, also, to note that the differences between the males and the females tended to disappear as the concentration of sugar reached a maximum, apparently in the neighborhood of 50 mgm. per 100 cc. Individual concentrations varied between 26 and 74 mgm. per 100 cc. Our

work revealed no relation between the length of time spent in the ice box and the concentration of blood sugar shown. The results are detailed in table 4, and summarized in table 2.

The reason for this excess of blood sugar in frogs kept at a reduced temperature is not clear though it might be suggested as a possibility that while the utilization of sugar at the lowered temperature is greatly diminished there is a lag in the modification of mobilization which allows a certain amount of accumulation in the blood. According to Loewit's work this excess may lead to an excretion of sugar in the urine.

SUMMARY

1. The average sugar content of the heart blood of ordinary small frogs (35.3 gm.) for February, March and April is 37 mgm. per 100 cc. of blood.

2. The sugar content of the males during these months is lower than that of the females and it is lower in the females with poorly developed eggs than in those with well filled ovaries.

3. Our results do not show any marked variation in the concentration of blood sugar during the onset of the breeding season of male frogs kept in a vivarium at 18°C.

4. Frogs kept at low temperatures for a few days show a higher average blood sugar concentration than do those kept at room temperature.

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A STUDY OF THE SUGAR IN THE BLOOD OF NORMAL PIGEONS

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There is an apparent dearth of literature on the carbohydrate metabolism in birds. The present paper is a study of the amount of sugar in the blood of the pigeon, as one of the phases of its carbohydrate metabolism. In order to lay the foundation for further work along this line an attempt has been made here to establish the normal amount of sugar in the blood of this bird. The pigeon was chosen because it is small, easy to obtain and to handle and, when the carrier pigeon is used, it offers obvious advantages for a study of fatigue.

Kausch (1) states that Claude Bernard (2) was the first to use birds for any form of experimentation. His *Mémoire sur le páncreas* tells of some experiments made in 1851 for the purpose of studying the rôle of the pancreas in birds. Ducks were used principally, although no values are given for their blood sugar. In 1894 Weintraud (3) made eleven determinations of blood sugar in ducks. The amount present varied from 0.177 per cent to 0.198 per cent. Kausch in 1896, in studying pancreatic diabetes in ducks and geese, found that in normal ducks the sugar content of the blood varied between 0.12 per cent and 0.18 per cent, the average being 0.14 to 0.15 per cent. Geese gave very much the same results. Starvation up to 72 hours did not affect the concentration of blood sugar noticeably. In birds of the same kind stated to be "sick," one with an infectious disease and others suffering from various pathological conditions, the blood sugar apparently did not vary from the normal. Saito and Katayama (4) in 1901 found the sugar content of fowls' blood to be higher than that of dogs' and rabbits'. Their average was 0.209 per cent. In 1912 Bierry and Fandard (5) recorded that the blood of birds, richest in free sugar, contains the smallest amount of combined sugar. In this statement they are probably making comparison with mammals. The kinds of birds used were not specified. Giaja (6) in 1912, working with fowls less than two

months old, and with those a year or less than a year old, showed that the younger birds had the higher percentage of blood sugar. The average for fowls of two months or less was found to be 0.201 per cent, for those from two months to one year, 0.182 per cent. Flemming (7) in 1919, in determining the effect of the extirpation of the pancreas upon ducks, found that in normal ducks the average per cent of sugar in the blood was 0.075 per cent. This value is only one-half as high as any of the other recorded values.

The results of these authors are summarized in table 1.

TABLE 1

Sugar in the blood of different birds

BIRDS	NUMBER	PERCENTAGE OF SUGAR			OBSERVER	REMARKS
		Minimum	Maximum	Average		
Ducks.	11	0.117	0.198	0.16	Weintraud	
Ducks.	11	0.05	0.098	0.07	Flemming	
Ducks.	14	0.12	0.18	0.15	Kausch	Normal ducks
Ducks.	5	0.11	0.15	0.136	Kausch	Fasted up to 72 hrs.
Ducks.	5	0.13	0.15	0.14	Kausch	Sick ducks
Geese.	6	0.12	0.16		Kausch	
Geese.	3	0.069	0.113	0.094	Flemming	Partial excision of pancreas
Fowl..			0.14		Bernard	Quoted from Weintraud
Fowl..	8	0.188	0.250	0.209	Saito and Katayama	
Fowl..	4	0.175	0.19	0.182	Giaja	Age, one year or less
Fowl..	4	0.2	0.24	0.201	Giaja	Age, two months or less
Raven.	1			0.27	Bang (8)	

MacLean's (9) method for determining the sugar in 1 cc. of blood has been in successful use in this laboratory during the past year, and was used in the present research. The small amount of blood required is an obvious advantage in working with small animals such as the pigeon.

The pigeons were removed from the cages and killed by decapitation as quickly and quietly as possible, in order to prevent any avoidable rise in the sugar content of the blood due to emotional disturbance. The blood was drawn in two samples, upon each of which a determination was made.

It is here seen that the sugar content of the blood runs from 160 mgm. to 430 mgm. per 100 cc. The average for the entire series is 225 mgm. per 100 cc. For some unknown reason the last three values are extremely high and, excluding these, the average is 185 mgm. per 100 cc.,

which is perhaps more nearly what one would expect to find in normal birds.

TABLE 2

Sugar content of pigeons' blood in milligrams per 100 cc.

NUMBER OF EXPERIMENT	WEIGHT OF PIGEON	AMOUNT OF SUGAR PER 100 CC. OF BLOOD.	REMARKS
	grams	*mgm.*	
19	310	160	
6	271	163	
21	315	163	
14	295	169	
12	307	169	
13	342	178	
10	307	189	
20	352	190	
7	342	208	
11	322	216	
15	252	239	
16	352	297	
18	355	390	
17	360	430	
Average...	313	225	
		185	Excluding experiments 16, 17 and 18

TABLE 3

Sugar content of oxalated and defibrinated blood

NUMBER OF EXPERIMENT	AMOUNT OF SUGAR PER 100 CC. OF BLOOD	
	Oxalated	Defibrinated
	mgm.	*mgm.*
17	430	430
18	390	390
5	300	276
19	160	160
20	190	190
21	163	163
22	121	121

In a few cases one of the samples of blood was oxalated while the other was defibrinated. The results are grouped in table 3. No difference appears in the sugar content of the oxalated and defibrinated blood except in the case of experiment 5.

The age of the birds in these experiments is not known. One bird (no. 22) was suffering from an injured leg, and the amount of sugar in the blood of this bird was considerably lower than in any of the others. Although this represents but a single observation, it is interesting to note that it is not in agreement with Kausch's generalization that the blood sugar is not modified in sick birds.

Probably the concentration of sugar in the rabbit is as high as in any of the mammals commonly used in the laboratory. Consensus of opinion places the normal value for this animal at about 100 mgm. per 100 cc. of blood. It will be seen that the sugar in the blood of birds is greatly in excess of that found in mammals. Though the explanation of this has not so far been worked out, it might not be out of place to suggest a possible correlation with the higher rate of metabolism necessitated by the higher body temperature. This suggestion has already been implied by Bierry (10). There is the further possibility of emotional hyperglycemia, and though considerable pains to avoid excitement were taken in handling the pigeons in the present research, we would not care to state that this might not have been a factor. It is interesting to note that Flemming succeeded in obtaining values for ducks which are of the same order of magnitude as is common in the mammals, and he alone speaks of taking particular care in this respect.

CONCLUSIONS

1. The concentrations of sugar reported for the blood of birds are much higher than is normal for mammals. In this the pigeon appears to be similar to other birds.

2. The average percentage of sugar in the blood of the pigeon is 185 mgm. per 100 cc.

3. The amounts of sugar in the oxalated and the defibrinated blood are the same.

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STUDIES ON THE VISCERAL SENSORY NERVOUS SYSTEM

VII. Skeletal Reflexes Induced by Stimulation of Visceral Afferent Nerves in the Frog and the Turtle

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As pointed out in our first paper (5), the aim of this work is the experimental analysis of the rôle of the visceral afferents in health and disease of man. In the case of the various forms of hypermotility and forced movements (parathyroid tetany, idiopathic epilepsy, spasmophilia, gastro-intestinal tetany, occupational tetany, tetany from intestinal parasites, etc.), the main efforts of analysis have in recent years been directed to the field of specific toxic substances (bacterial and metabolic) acting directly on the nervous tissues. We do not underestimate the importance of the progress made in this direction, especially in the case of parathyroid tetany. But at the same time the possibility of functional upset of normal reflexes should not be neglected. Of course, Jacksonian epilepsy stands in a group by itself, in being definitely proven to be of nervous or cerebral origin.

The view that visceral afferent impulses play a part in some forms of forced or involuntary movements in man is not new. But the data supporting this view are fragmentary and inconclusive. It would seem that the first step is the determination of the reflex connections that exist in normal animals between the various viscera and the skeletal neuro-muscular system. If such reflex connections do not exist in health, it is not likely that they are developed by disease. On the other hand, if they do exist in health, there are several ways in which these viscero-skeletal reflexes may be exaggerated in disease. We may have impairment of the inhibitory influence of the brain centers over the lower reflexes. We may have an increase in the excitability of the visceral afferents as well as actual exaggeration of the normal stimuli. Or, bacterial and abnormal metabolic products may act on particular reflex nexuses similar to the general action of strychnine, so that normal

afferent impulses produce abnormal central effects. It would seem that these various possibilities are capable of at least proximal experimental attack.

LITERATURE

Anatomists, on the basis of their histological investigations, have furnished ample evidence that the extrinsic nerves of the various thoracic and abdominal viscera contain afferent fibers. But neither they nor contemporary physiologists have made any systematic experimental investigation of the physiological effect which impulses, carried over these afferent fibers might have on general body musculature either under strictly normal conditions, or as the results of experimental stimulation of the viscera themselves and the nerve trunks with which they are supplied. As was pointed out in our first paper (5), even monographs dealing with the physiology of the sympathetic nervous system deal only with the efferent fibers contained in its visceral branches. Reference hand books of physiology such as Nagel (12) state that the stimulation of the white rami which have their distribution in the thoracic and abdominal viscera elicit reflex movements and a rise of general blood pressure. When, however, we searched for the experimental work describing skeletal reflexes from the stimulation of the viscera themselves or their nerves, we were forced to content ourselves with but few references bearing directly on our work.

According to Buch (4), Magendie found that evulsion of the abdominal sympathetic caused no pain but muscular movement on the part of the animal. General statements appear here and there in the literature (especially text books) that there are no sensory fibers in the cervical sympathetic nerve. Magendie (11), among others, states that evulsion of the cervical ganglia can be performed in dogs and horses "without the animals appearing conscious of the injury." Goltz (7), in repeating some observations of Budge, found that the skeletal reflexes can be elicited by mechanical stimulation (pinching) of the heart of the frog. He rarely obtained movements of the animal from stimulation of the ventricle (cutting, crushing, cauterizing or burning). The application of dilute acetic to the sinus region immediately caused movements of the entire body which lasted for some time. Since these skeletal reflexes were abolished after section of the vagi, Goltz concluded that the afferent fibers were carried by the vagi.

More recently Sano (15) extended the original observation of Goltz by showing that weak acetic (0.5 per cent sol.) when applied to the

stomach and duodenum of a normal frog likewise induced generalized convulsions. We find in this article the first notation of the fact that cutting of the stomach and intestine elicits general body movements. In an article published the following year, Sano (16) stated that in normal frogs skeletal reflexes can be elicited from chemical and mechanical irritation of the heart and stomach. He found the other internal organs to be relatively insensitive to chemical stimulation and to cutting. These skeletal reflexes elicited from the viscera were abolished by dosages of strychnine sulfate which raised the excitability of the animal far above the normal as determined by stimulation of the skin, muscle, and the peritoneum.

Several years later Boruttau and Braun (2) showed that in the frog the manipulation of a loop of gut rendered inflamed and hyperemic by previous ligation induced strong defensive movements of the entire body. These investigators also fastened a loop of intestine in the laparotomy wound of dogs operated under morphine. Subsequent mechanical or electrical stimulation of this loop caused crouching movements of the animal involving movements of the head and neck.

Every physiologist is familiar with the controversial literature on the sensibility of the abdominal viscera which followed the appearance of Lennander's (10) paper on the subject. Kast and Meltzer (9) in their experimental criticism of Lennander's position allude to the presence of afferent nerve fibers in the sympathetic nervous system other than pain fibers whose stimulation elicits cardio-inhibitory, vasomotor and respiratory reflexes. No direct reference is made in this article or others to skeletal reflexes resulting from stimulation of abdominal viscera except that these authors considered movements of the head, body and extremities together with whining as evidence following such stimulation, that the animal experienced pain if these reactions began immediately on application of the stimulus and stopped with cessation of the irritation. Haffter (8), Nasse (13) and van Braam-Houckgeest (3) likewise found earlier in the century that reflex movements in the extremities and pain followed isolation, section, stimulation or evulsion of the nerves supplying some of the abdominal viscera.

It is clear, however, that such motor response may be the result of pain or arise quite independently of it. This possibility was recognized by Kast and Meltzer for they state that an acceleration of respiration and contraction of the abdominal muscles without the other signs of pain were considered as simple reflex effects which were possibly not accompanied by pain.

Palmer (14) more recently failed to influence the onset or severity of the tetany of parathyroidectomized animals by irritation of the gastro-intestinal tract, although he quotes from the literature evidence to the effect that some forms of tetany in man may arise from stimulation of the gastro-intestinal tract. In a recent paper Alvarez (1) presents clinical data collected by himself and others purporting to show that many if not all of the symptoms and signs of so-called autointoxication can be attributed to afferent impulses coming from a distended, overactive or wrongly acting bowel, particularly in sensitive people.

METHODS

Frogs and turtles were used exclusively in this study. The animals were rapidly decerebrated, care being taken to avoid hemorrhage. Following decerebration (5 minutes to 3 days) the frogs were placed on their backs on a frog board and kept in that position by the frog clips placed about the extremities. A median abdominal incision carried forward to the base of the heart exposed the thoracic and abdominal viscera. Ordinary pins were passed through the edges of the abdominal wall and nailed to the frog board. The viscera were thus held in shallow basins whose sides were the abdominal walls themselves. The clips were now removed from front and hind legs. Under such conditions the frogs fill their lungs at regular intervals as during normal respiration. The exposed viscera were kept moist with Ringer's solution. The viscera were then gently lifted up by means of a hook made of a bent pin. After stimulating the various viscera or the mesentery with a tetanizing current and noting the results, the various viscera were successively grasped with the fingers and squeezed. Following this mild mechanical stimulation, the organs were stretched, cut with scissors, and crushed between the jaws of a hemostat.

In this type of preparations all injections of Ringer's solution or dilute solutions of strychnine were made intravenously through a cannula inserted in the anterior abdominal vein. A few frogs were strychninized by subcutaneous injection of the drug in Ringer's solution immediately following decerebration. In order to determine whether or not the movements of the animals following stimulation of the various thoracic and abdominal viscera were skeletal reflexes of spinal origin or defensive movements due to brain impulses, we transected the cord below the medulla immediately after decerebration.

Following decerebration some of the turtles were attached to the turtle stand in a manner fully described in a previous article (6). The thoracic and abdominal viscera were exposed through a dorsal window cut through the shell with a bone-cutting forceps. Artificial respiration was given, when necessary, through a cannula tied in the tip of the exposed lung. All organs were stimulated successively as in the frogs. When it was desired to note the effects of strychnine on skeletal reflexes elicited by various forms of stimuli applied to the viscera the drug was injected intravenously through a cannula inserted in the external jugular vein. In a few instances we sectioned the cord below the medulla to determine what effect such a transection had on the skeletal reflexes elicited from a stimulation of the abdominal organs on the assumption that this procedure would decide whether or not the movements of the animal were the result of pain.

Some turtles, following decerebration, were placed on their backs. After introducing a cannula into the trachea for purposes of artificial respiration the plastron was removed. Under such conditions the organs were more readily accessible for stimulation. It is equally plain, however, that such animals were not in the best possible physiological condition because of unavoidable hemorrhage and the traumatic shock brought on by removal of the carapace.

In one instance we anesthetized a turtle with ether without previous decerebration and made a window through the plastron. This window was closed with medium weight "cellosilk" (celluloid) kept in place with some bone wax. On the following day when the animal had fully recovered from the anesthetic the abdominal organs were handled through this ventral window and were stimulated in various ways.

In the case of electrical stimulation the threshold stimulus for a given organ or viscus is expressed in the distance in centimeters of the secondary coil from the primary coil of an ordinary laboratory induction machine.

RESULTS

Frogs

Normal animals. The following typical experiments may be cited:

January 23, 1920. Animal fixed dorsal side down and viscera exposed by median incision immediately after decerebration. Stimulation with a moderately strong tetanizing current of the small and large intestines, intestinal mesentery and the central end of the splanchnic nerves causes escape movements and struggling involving all the skeletal muscles, temporary cessation of respira-

tory movements in expiration, and a forward movement of the tongue. The forward movement of the tongue appeared to be tetanic or clonic in character. Stimuli too weak to induce the general skeletal reflexes were still capable of producing the above effect on respiration and tongue.

January 26, 1920. Frog, decerebrated on the previous day, fixed dorsal side down and viscera exposed. Stimulation with moderately strong tetanizing currents of the wall and neck of urinary bladder caused movements of all extremities, head and neck, cessation of the respiration in the expiratory stage, and forward movement of the tongue.

The same stimulation applied to rectum, large and small intestines induced similar reflexes. Rubbing the mucous membrane and stretching the walls of the large intestine and rectum induced similar body reflexes. As the preparation deteriorated the movements of the extremities were the first reflexes to fail on stimulation of the urinary bladder and the gut.

October 20, 1920. Frog decerebrated 30 minutes prior to being fixed dorsal side down and the viscera exposed.

1. Electrical stimulation (moderately strong tetanizing current) applied to the walls of the stomach, small intestine, large intestine, urinary bladder, pedicle of the spleen, pedicle of the gall bladder, and intestinal mesentery induced escape movements of the extremities, movements of the head and neck, cessation of the respiration, forward movement of the tongue, and closure of the eyelids.

2. The ileum was hyperemic and the skeletal reflexes were more readily induced from this region than from the normal regions of the gut.

3. Electrical stimulation of the ovarian mesentery caused inhibition of the heart but no skeletal reflexes.

4. Mechanical stimulation (stretching, cutting, pinching with forceps, rubbing between fingers) of the esophagus, stomach, large and small intestines, urinary bladder, gall bladder, lungs, induced the same skeletal reflexes as electrical stimulation of these organs. Rubbing or pressing these organs between the fingers induced skeletal reflexes practically as vigorous and prolonged as similar handling of the toes.

5. The respiratory inhibition, tongue protrusion and head and neck movements are induced with weaker stimuli (electrical or mechanical) than the movements of the extremities, and the latter reflexes disappear first as the preparation deteriorates from visceral exposure and traumatic shock.

6. The skeletal reflexes could be induced from the oviducts (electrical or mechanical stimuli). Skeletal response from the kidneys was uncertain on account of the difficulty in stimulating these organs without traction on or escape of the current to other parts.

These experiments show conclusively that appropriate stimulation of the afferent nerves of the alimentary tract, the lungs, the urinary bladder, the gall bladder and the mesentery induces reflex contractions of the entire skeletal muscle system, provided the nervous system of the animal is not depressed by anesthetics or shock. In the case of the hollow viscera (gut, lungs, urinary bladder and gall bladder) mechanical stimuli (pressure, stretching, cutting) are more efficient than

electrical stimuli in producing the skeletal reflexes. But in sufficiently sensitive preparations the tetanizing current applied to these organs in a strength which is easily tolerated on the observer's tongue is sufficient to induce struggling and escape movements.

As a rule the skeletal reflexes do not persist for any length of time after cessation of the visceral stimulation. And unless the preparation is in prime condition the reflexes fail after a few seconds even though the visceral stimulation is continued. Throughout this work we have been impressed by the quick failure or onset of "fatigue" of these reflex mechanisms, the reflex into the extremities showing the quickest fatigue and earliest failure under the conditions of the work. This indicates a complex path of conduction in the visceral afferent system.

Influence of ether anesthesia. Only a few experiments were made on the influence of general anesthesia on the viscero-skeletal reflexes, as the results were constant and diagrammatic. In fact, one might have predicted the results in advance of the tests. The following is a typical experiment:

October 22, 1920. Frog decerebrated 5 hours previously, fixed on the board, dorsal side down and ether applied by means of a cotton wad held close to the nares, until the skeletal reflexes were nearly abolished. The viscera were then quickly exposed.

1. Electrical or mechanical stimulation of the stomach, intestines, urinary bladder, gall bladder and mesentery failed to induce reflexes to the heart, the respiratory mechanism and the skeletal muscles, although good skeletal reflexes (unilateral and crossed) were present.

2. After the return of vigorous respiratory and some voluntary movements the above visceral stimulations evoked the usual cardiac, respiratory and skeletal reflexes. These reflexes were feeble at first, and increased in intensity with the gradual recovery from the ether.

It is clear that general anesthesia has a greater depressor influence on the skeletal reflexes evoked from the visceral afferents than on those induced by stimulation of the cutaneous afferents.

The influence of strychnine. The influence of strychnine on the skeletal reflexes induced by the stimulation of visceral afferent nerves was studied by three modes of approach.

1, Decerebrated frogs were poisoned with strychnine to the point of moderate convulsions before exposing and stimulating the viscera. 2, The appropriate dose of strychnine was injected into the abdominal vein after exposure of the viscera. 3, Using electrical stimulation exclusively and fixed points in the viscera where the stimulus was

applied the viscero-skeletal reflexes were recorded at intervals for several hours before giving the strychnine intravenously, in order to control the changes in viscero-skeletal reflex excitability that may take place from exposure, hyperemia and hyperexcitability of the visceral organs, or shock from unavoidable visceral trauma during a prolonged experiment. The following experiments may be cited:

October 20, 1920. Normal frog. Three subcutaneous injections each of 2/100 mgm. strychnine sulfate in the course of 45 minutes. Frog decerebrated 30 minutes after the first injection. Moderate strychnine spasms appeared 60 minutes after the first injection. The animal was then prepared in the usual manner and the viscera stimulated.

Stomach. Electrical stimulation of wall of stomach and of gastric mesentery caused convulsions, cardiac and respiratory standstill. Rubbing, pinching and crushing stomach caused violent convulsive spasms.

Small intestines. Touching intestine with forceps or electrodes caused temporary cardiac inhibition, but no skeletal response. Electrical stimulation of intestine and intestinal mesentery, and pinching or rubbing the intestine caused convulsions, together with heart and respiratory inhibition.

Large intestine. Electrical and mechanical stimulation caused convulsions, cardiac and respiratory inhibition.

Urinary bladder. Electrical stimulation, rubbing, cutting and crushing induced skeletal convulsions.

Gall bladder. Pressure, rubbing and pinching induced skeletal convulsions.

Lungs. Electrical stimulation or rubbing apex of one lung between fingers induced skeletal convulsions.

October 25, 1920. Frog decerebrated October 22, fixed on board and viscera exposed as usual. The minimal strength of the tetanizing current causing skeletal reflex response or strychnine convulsions on application to the viscera was then determined, in the terms of centimeters distance of secondary from primary coil.

	cm.
10:45 a.m.	
Small intestine	10
Stomach	12
Urinary bladder	11
10:55–11:15	
Intravenous injection of 6/100 mgm. strychnine sulfate	
11:25 a.m.	
Small intestine	12
Stomach	14
Urinary bladder	15
11:50 a.m.	
Small intestine	12
Stomach	13
Urinary bladder	13

12:34 p.m.
Small intestine..... 12
Stomach..... 12
Urinary bladder..... 15
1:30 p.m.
Small intestine..... 19
Stomach..... 20
Urinary bladder..... 20

The tests by electrical stimulation were discontinued, and various types of mechanical stimulation employed with the following results:

Urinary bladder, stretching, cutting, crushing = strychnine spasms.
Small intestine, stretching, cutting, crushing = strychnine spasms.
Large intestine, stretching, cutting, crushing = strychnine spasms.
Stomach, stretching, cutting, crushing = strychnine spasms.
Gall bladder, stretching, cutting, crushing = strychnine spasms.
Lung, rubbing tip between fingers = strychnine spasms.
Heart, cutting and crushing ventricle and auricle = strychnine spasms.
Sinus, application of 1 per cent acetic acid = strychnine spasms.

In this experiment there was apparently a definite increase in the reflex excitability from the viscera as measured by the minimum strength of the tetanizing current, but it is not clear that this is due to the strychnine. The various viscera were moved and lifted by means of a probe or a pair of forceps. While care was taken to inflict the minimum amount of trauma, it is certain that some injury was done to the tissues under investigation, and the rise in excitability might have been due to this factor.

In harmony with the results in the experiments where the strychnine hyperexcitability was induced in the animal before exposing the viscera, there was rapid fatigue of the skeletal reflexes following visceral stimulation, and the resistance to reflex response from the visceral stimulation increased in the following order, cardiac inhibition, respiratory inhibition and tongue protrusion, head and neck movements, movements of the extremities.

October 25, 1920. Frog decerebrated October 22. Animal fixed and viscera exposed as usual October 25, 3:00 p.m. The viscera were handled in the gentlest manner by means of a bent needle, thus avoiding pressure and rubbing. The reflex excitability was tested every 30 minutes, care being taken to apply the electrodes to the same spot on the respective organs in each set of tests. In each group of tests the start was made with a tetanizing current too feeble to give skeletal response, and the current gradually increased. In this way any increase or decrease in excitability should have been detected. Throughout the experiment the viscera were kept covered with Ringer's solution, and the skin prevented from drying by surrounding the animal with cotton moistened with tap water. The circulation and respiration continued vigorous throughout the experiment. The intravenous injection of 6 cc. Ringer's solution was made as a control on the intravenous injection of strychnine, as the 0.06 mgm. strychnine sulphate was introduced in 6 cc. Ringer's. The results are summarized in the following table, only the skeletal response (legs) being recorded. The respiratory and cardiac reflexes were invariably evoked by weaker stimuli than those required for the leg reflexes.

Strength of tetanizing current required to cause reflex response or strychnine spasm of extremities (cm. distance of secondary coil)

ORGAN STIMULATED[a]	3:00 P. M.	3:30 P. M.	4:00 P. M.	4:30 P. M.	5:00 P. M.	5:30 P. M.	6:00 P. M.	10:00 A. M.
Urinary bladder	11	9	9	9	9	10	10	9
Small intestine	12	10	10	10	10	12	12	10
Large intestine	11	10	10	11	11	10	10	10
Stomach (pyloric end)	11	10	10	9	9	10	10	10

ORGAN STIMULATED[b]	10:30 A. M.	11:00 A. M.	11:30 A. M.	12:00 A. M.	12:30 P. M.	1:00 P. M.
Urinary bladder	10	11	10	10	11	10
Small intestine	11	11	11	11	12	12
Large intestine	11	11	11	11	10	12
Stomach (pyloric end)	10	10	9	9	9	9

a. Intravenous injection of 6 cc. Ringer's solution.

b. Intravenous injection of 0.06 mgm. strychnine sulphate in 6 cc. Ringer's solution.

As in previous experiments, weaker stimuli called forth the cardiac, respiratory, tongue and head reflexes. The leg responses evoked after the strychnine administration (11 a.m.–1 p.m., Oct. 26) were the typical extensor spasms.

This experiment seems to show that strychnine produces little or no change in reflex irritability from the viscera. The visceral excitability is certainly not reduced or abolished as claimed by Sano (15), (16).

Experiment November 8–9, 1920. Spinal cord transected just below the medulla 3:40 p.m. Viscera exposed and the threshold for the spinal reflexes determined; 0.06 mgm. strychnine sulphate was then injected intravenously and the visceral irritability tested up to 6 p.m., when the preparation was left well protected against drying until 10 a.m. the following day. This dose of strychnine was not sufficient to tetanize the respiratory muscles. The circulation continued in good condition till the end of the experiment, 11 a.m., November 9. In this experiment only the urinary bladder and the small intestines were used as points for the visceral stimulation.

ORGAN STIMULATED	4:00 P. M. *b*	4:15 P. M.	4:30 P. M.	5:00 P. M.	5:30 P. M.	6:00 P. M.	10:00 A. M.	10:30 A. M.	11:00 A. M.
Urinary bladder	10	11	9	10	10	11	10	11	11
Small intestine	10	10	9	10	10	10	10	11	12
Skin hind leg	11	16	11	11	11	13	12	13	12

b. Intravenous injection of 0.06 mgm. strychnine sulphate in 6 cc. Ringer's. Typical strychnine spasms developed within 3 minutes after the injection.

Beginning with the first test after the strychnine injection at 4:14 p.m., the skeletal responses to the visceral stimulation were typical extensor spasms. On the morning of November 9 the preparation was still under the influence of the

strychnine to the extent that the extensor spasms predominated in the skeletal responses. The viscera were hyperemic. Defensive and escape movements of the hind legs were induced by the slightest touch, pressure or rubbing of the stomach, the small and large intestines, the urinary bladder, and the gall bladder. Putting a clamp on the small intestine or merely transecting the small intestine with a pair of sharp scissors induced prolonged and violent leg movements.

It seems that as tested by the tetanizing current, skeletal reflexes from the viscera are purely a question of a strength of stimulus sufficient to initiate activity in the afferent nerves or nerve endings in the respective organs, and that the excitability of these visceral afferents are not changed by strychnine. Of course, the skeletal response evoked by the minimum visceral stimulation is greater in the frogs poisoned with strychnine. But the fatiguability appears to be greatly increased. In fact, one may induce fatigue to the point of complete failure of response by a type of stimulation of the viscera that at no time evokes the skeletal reflexes. For example, if in a strychninized preparation the bladder threshold for the skeletal response is with the secondary coil at 10 cm., provided an interval of 1 to 5 minutes elapses between each stimulation period, we may start with the secondary at 20 cm. distance and work up by stages to 10 or even 0 and no skeletal response is obtained at any point. That is to say, stimuli too feeble to induce the reflex are still capable of so fatiguing part of the reflex path that even the strongest stimuli, electrical or mechanical, fail to pass through to the final motor path.

Viscero-spinal reflexes in the spinal frog. In these experiments the spinal cord was transected just below the medulla, and the brain was left intact. This operation usually suspended the respiration for a few minutes. On the return of respiratory movements the animals were fixed dorsal side down and the viscera exposed and stimulated in the usual way.

Experiment, November 3, 1920. Spinal transection 9:30 a.m. Skeletal reflexes from the viscera were induced by the following strength of the tetanizing current.

ORGAN STIMULATED	10:00 A. M.	10:30 A. M.	11:00 A. M.	11:30 A. M.
Urinary bladder	14	11	10	9
Small intestine	14	12	11	10
Stomach	13	11	10	9
Large intestine	13	10	9	9

In this spinal preparation the skeletal movements following the visceral stimulation were distinctly less vigorous than in decerebrated preparations. The rapid decline in the reflex excitability was probably due to partial failure of the circulation and consequent asphyxia.

Experiment, November 6, 1920. Spinal cord transected just below the medulla, 9:30 a.m. Skeletal (leg) movements occurred with the following strength of tetanizing current.

ORGAN STIMULATED	10:00 A. M.	10:30 A. M.	11:00 A. M.	11:30 A. M.	12:00 M.	2:00 P. M.
Small intestine	10	9	8	9	9	11
Mesentery, small intestine	9	9	8	9	9	11
Large intestine	9	8	8	9	9	11
Rectum	9	8	8	9	9	11
Urinary bladder	8	8	8	9	9	10
Stomach	9	7	8	9	9	10

In this experiment the skeletal movements caused by the visceral stimulation were less vigorous than in decerebrated preparations.

Experiment, November 8, 1920. Spinal transection just below the medulla at 11:40 a.m. One hour later the viscero-skeletal reflexes were evoked with the secondary coil at 11 cm. distance. Two hours after the transection it required a slightly stronger stimulus (10 cm.). But the skeletal movements were less vigorous than in the decerebrated preparations.

In a second experiment on this date the spinal transection was made 2 hours before exposing and stimulating the viscera. Feeble responses of the extremities followed visceral stimulation with the secondary at 11 cm. Thirty minutes later the several organs required the strength of current represented by the secondary coil at 11 and 8.

The experiments with purely spinal preparations were undertaken with the view of finding out whether the stimulation of the viscera induces pain sensation parallel with the purely reflex effects on the skeletal system. The reflexes are, at least in part, purely spinal, and to that extent independent of conscious pain processes. The fact remains, however, that the viscero-spinal reactions are less vigorous in the purely spinal preparations. This may be due to a degree of "spinal shock" not present in the decerebrated preparations but there is also the possibility of bulbar and midbrain pain processes as a cumulative factor.

Turtles

Animals decerebrated and viscera exposed by removal of the plastron. Artificial respiration.

Experiment, January 25, 1920:

Urinary bladder. Traction on empty bladder, distention of bladder with air and stimulation of the neck of the bladder with a moderately strong tetanizing current caused movements of head and neck, front and hind legs, and tail.

Large intestine and rectum. Stimulation with moderately strong tetanizing current, and mechanical rubbing of the mucosa of the rectum with a catheter caused violent movements of the tail, head and neck, and front and hind legs.

Experiment, January 29, 1920:

Urinary bladder. Inflation of small balloon (introduced through slit in bladder wall), electrical stimulation, stretching and crushing cause movements of head and neck, all extremities, and contraction of the sheets of muscle in the flanks and dorsal region of the abdominal cavity that act as expiratory muscles. These expiratory muscles contract reflexly on bladder stimulations that are too weak to cause movements of head, neck and legs.

Large intestines and rectum. Inflation of balloon in rectum, in cloaca, and in lateral pouches of cloaca; and electrical stimulation of the large intestine caused general body movements (extremities, head, tail).

Oviducts. Inflation with balloon and electrical stimulation did not induce skeletal reflexes.

Abdominal sympathetic nerves. Stimulation tetanizing current of central end of nerves 1, 2, 3 and 4 (6, fig. 2) caused contraction of expiratory muscles and general body movements.

Cervical sympathetic nerve. Tetanization of central end of the cervical sympathetic nerves did not induce skeletal reflexes.

Animals with intact brain. Experiment 1, February 9–10, 1920. Under general ether anesthesia a large opening was made through the plastron, leaving the peritoneum intact. The opening was closed by "cellosilk" and wax, and the animal kept for 24 hours, to completely recover from the ether. On the following day the viscera were exposed by dissecting away the soft parts of the abdominal wall in the area where the calcareous part had been removed under anesthesia, and the following organs stimulated:

Urinary bladder. Moderate distention by inflation of balloon introduced through a small slit in the wall caused movements of tail and movements of penis. Very strong inflation of the balloon caused slight movements of head and neck and front legs.

Electrical stimulation of the bladder with moderately strong tetanizing current caused movements of tail and head and neck, and slight movements of the extremities. When the horns of the bladder were stimulated the head and neck movements were usually toward the side of the stimulation.

Large intestine and rectum. Moderate distention with balloon in lumen caused prompt movements of the tail. Very strong pressure in the balloon usually induced some movements of head and extremities. Tetanization gave more vigorous movements of legs and head than the balloon inflation.

The spinal cord was now transected in the mid-dorsal region, and the stimulation of the pelvic viscera repeated with the following results:

Urinary bladder. Traction on horns, tetanization, crushing of walls caused violent defensive movements of hind legs and twisting and turning of tail with final position in midline.

Large intestine and rectum. Balloon inflation, electrical stimulation and crushing caused violent movements of hind legs and tail, persisting longer than the period of stimulation. If the leg and tail movements were going on, the stimulation of the large intestine or the urinary bladder caused temporary inhibition of the movements. The usual order of events in the hind leg movements following the stimulation of bladder and large intestine was an initial relaxation followed by vigorous and rhythmical brushing movements evidently "defensive" in character.

The same strength and character of visceral stimulation causes less vigorous skeletal reflexes in animals with the brain intact than in decerebrated animals, and after high thoracic spinal transection the movements of the hind legs and tail on stimulation of the pelvic viscera are more vigorous than in decerebrated preparations. These facts are capable of two interpretations: *a*, The intact brain shunts the visceral sensory impulses away from the skeletal reflex pathway; or *b*, decerebration (as well as spinal transection) increases the reflex excitability peripheral to the lesion.

Animals decerebrated and pelvic viscera exposed by removing dorsal shell on one side. The animals prepared in this way were secured on the turtle stand in their normal posture or ventral side down, head, neck and extremities being free to execute any movement.

Experiment, February 11, 1920:

Urinary bladder. Distention by balloon as well as electrical stimulation induced violent escape movements of fore and hind legs. If the inflation of the bladder was extensive and was maintained, there were usually only initial skeletal movements, followed by a flaccid condition of the legs (inhibition of the tonus). In these cases sudden release of the pressure in the bladder balloon leads to escape movements of front and hind legs.

Large intestine and rectum. The types of stimulation used on the bladder gave practically the same skeletal reactions from the large intestine and rectum. The inhibition of leg movements in progress was usually not so marked with electrical stimulation as from inflation with a balloon.

Decapitated animals. Viscera exposed by removing plastron.

Experiment, February 14, 1920: Animal decapitated (including section of both vagi) 30 hours prior to these tests, artificial respiration being maintained part of this time.

Urinary bladder. Tetanization, pinching and stretching initiated movements of tail, front and hind legs. If these reflexes were going on as a result of a previous stimulation, a subsequent stimulation of exactly the same character, intensity and duration may inhibit the movements during the stimulation.

Large intestine and rectum. Tetanization or pinching caused movements of tail and legs, the movements of the hind legs and tail being the most vigorous.

Skeletal reflexes from the lungs. In decerebrated turtles in good reflex condition mechanical or electrical stimulation of the lungs invariably causes movements of head and neck, and escape movements of the legs. Mechanical stimulation is the most efficient. Thus pressing the tip of one (collapsed) lung between the fingers, or ligating the lung tip about a cannula invariably causes escape movements of the turtle. This was observed repeatedly in our previous work on lung automatism and lung reflexes in the reptilia. When electrical stimulation is used it requires about the same strength and duration of the tetanizing current to cause skeletal reflexes from the lungs as from the alimentary tract.

Skeletal reflexes from the cervical sympathetic nerves. In most of our turtle preparations stimulation of the central end of the cervical sympathetic nerves failed to induce skeletal reflexes. Even the most easily elicited expiratory reflex could not be elicited. This is in harmony with the prevalent view that the cervical sympathetic is a purely efferent nerve.

But in a number of preparations the stimulation caused some skeletal response, including the expiratory reflex. So far as we are able to determine these cases of positive results were not obtained under conditions that would favor escape of the stimulus (tetanizing current) to adjacent afferent nerves any more than did similar stimulations that had no effect. Vasomotor reflexes are occasionally obtained from this nerve (17).

We must be dealing with exceedingly few afferent fibers in the cervical sympathetic, in which case response or no response is a question of degree of reflex excitability of the preparation or the presence of afferents in that nerve is inconstant in the same species. It may be a question of a few spinal or muscular afferents occasionally joining the cervical sympathetic trunk on the course to their peripheral distribution.

The influence of strychnine:

Experiment, November 1–4, 1920. Vigorous turtle, weighing about 2 kilos. Animal decerebrated, secured on turtle stand, and viscera exposed by removal of part of dorsal shell on the left side, great care being taken to have the visceral nerve connections intact. There was very slight hemorrhage in preparation, and the circulation remained good throughout the experiment, especially during the first 2 days.

The electrodes were applied at the same points of the respective organs in the several tests, and care was taken to handle the viscera in the gentlest manner so as not to induce changes in excitability from trauma and hyperemia. Except during the tests the hole in the dorsal shell was covered with cotton moistened with Ringer's solution. During the night the preparation was kept in a cool room, the temperature ranging from 8°–12°C.

The results are summarized in the following table:

ORGAN STIMULATED	STRENGTH OF TETANIZING CURRENT REQUIRED TO CAUSE REFLEX RESPONSE OR STRYCHNINE SPASMS OF EXTREMITIES (CM. DISTANCE OF SECONDARY COIL)										
	November 1						November 2			November 4	
	2:30 p. m.	3:00 p. m.	3:30 p. m.	4:00 p. m.	4:30 p. m.	5:00 p. m.	10:00 a. m.	10:30 a. m.		11:00 a. m.	10:00 a. m.
Urinary bladder	6	6	7	*a*	7*b*	7*c*	6	6	*e*	6	6
Small intestine	8	7	7		6*b*	6*c*	7	7		7	7
Large intestine	7	7	7		6*c*	6*c*	7	7		7	7
Rectum	10	9	9		6*c*	6*c*	8	8		8	7
Intestinal mesentery	8	8	8		6*c*	6*c*	7	7		7*f*	7g

a. Intravenous injection of 7.5 mgm. strychnine sulphate in 10 cc. Ringer's solution.

b. Inhibition of the clonic convulsions (temporary).

c. Augmentation of clonic convulsions and tetany.

d. No spontaneous clonic convulsions, but typical extensor strychnine spasms from cutaneous stimulation.

e. Transection of spinal cord in the neck.

f. Strychnine spasms also produced by pinching bladder, small and large intestine and rectum.

g. Crushing these organs also evoked movements of the extremities.

In these tests we always started with a strength of the tetanizing current too feeble to cause skeletal reflexes and gradually working up until definite movements of the legs were induced by tetanization of the respective organs for periods of 5 seconds.

As in the frog, respiratory reflexes (expiration) are induced from the viscera with stimuli too feeble to cause leg movements, and even with an adequate stimulus the latent period for the leg reflexes is usually longer than for the respiratory reflex, so that the latter precedes the leg movements, almost without exception.

It will be noted from the table that during the first two hours after administration of the large dose of strychnine there seems to be some reduction of the viscero-skeletal reflex excitability. But inasmuch as the preparation executed almost continual clonic spasms during this period it was difficult to secure reliable observations on the reflex response. We are inclined to interpret the slightly stronger stimuli required at 4:30 and at 5:00 p.m. on November 1 as due to fatigue of the preparation, rather than to specific decrease in visceral sensibility. On the following day (Nov. 2), with disappearance of spontaneous clonic convulsions, the same strength of the visceral stimulation evoked

strychnine spasms, as sufficed to induce leg movements before the strychnine injection.

It is therefore apparent that strychnine has little or no effect on the threshold of viscero-skeletal reflex excitability. But these reflexes are more rapidly fatigued when the preparation is poisoned with strychnine.

COMMENTS ON THE RESULTS

It is evident that the afferent nerve fibers of the hollow viscera (urinary tract, gastro-intestinal tract, gall bladder, lung) make reflex connections with the skeletal motor system. The skeletal reflexes are evoked from the viscera in purely spinal preparations, and hence are not primarily responses to central brain processes evoked by the visceral stimulation. But the fact that in good preparations, that is, preparations showing minimum shock, the reflexes are essentially defensive or "escape" movements may indicate synchronous pain processes when the brain remains intact.

The skeletal reflexes induced by visceral stimulation are characterized, on the whole, by long latent periods and quick fatigue. This indicates a more complex synaptic system either in the spinal cord or on the visceral afferent paths, than in the case of the skeletal reflexes induced by stimulation of the extero-ceptors. This calls to mind the long controversy of the anatomists on the structure of the visceral afferent path, some holding that it is made up of at least two neurones with synaptic connections in the spinal ganglia or in the sympathetic ganglia along the spinal column.

At least for the hollow viscera so far investigated mechanical stimulation is more efficient than electrical stimulation in inducing skeletal reflexes.

Strychnine neither raises nor lowers the viscero-sensory threshold for the skeletal reflexes. The afferent depression by large doses of strychnine that cause more or less continuous clonic convulsions is probably due to "fatigue" in the spinal cord. In preparations poisoned with strychnine the visceral stimulation induces the same kind of skeletal hypermotility as cutaneous stimulation. But the reflexes caused by the stimulation of the visceral afferents are more readily fatigued under the influence of strychnine.

The inhibitory control over the extero-ceptive spinal reflexes by the brain in normal animals is a well-established fact. The higher centers appear to exercise a similar inhibitory control over the spinal skeletal

reflexes caused by visceral stimulation. This may be the mode of preventing the visceral afferents from disturbing the skeletal reflex system in the normal individual. The fact has also some bearing on the problem of visceral pain, for it would seem that if the stimulation of the normal viscera induced pain, the skeletal response should be more violent in animals with the brain intact than in purely spinal or decerebrated preparations. But an animal like the turtle may not be a suitable object to work out this relation because of its special defensive reaction (withdrawing under the protection of the shell).

On the whole we can state that in the frog the spinal transection beneath the medulla diminished the vigor of the skeletal response from stimulation of the abdominal viscera much more than a similar spinal transection in the turtle in spite of the fact that such a transection abolished external respiration in the turtle permanently and had but little effect on the external respiration of the frog. It is possible, of course, that the asphyxia occurring promptly in the turtle might account for the increased vigor of the response, after spinal transection as compared with the frog which breathes in a normal fashion after cord transection. We endeavored to rule out asphyxia by ventilating the lung of the turtle artificially. It seems more probable that the difference in the response in the frog and the turtle following spinal transection is a question of shock. In the turtle spinal shock is less pronounced than in the frog. The depression of reflex excitability for visceral stimulation following spinal transection should therefore be less pronounced in the former animal. This was found to be the case.

A series of observations on a strychninized frog convinced us that the functional activity of the cord could be depressed by stimulating any afferent nerve (cutaneous or visceral) with subminimal stimuli. If, for example, skeletal reflexes could be unfailingly evoked by a short tetanization of the bladder with the secondary coil at 10 cm. distance from the primary (threshold stimulus), this strength of the current or one many times stronger applied to the bladder failed to elicit skeletal reflexes if we had previously stimulated the bladder in rapid succession with the secondary coil at 15, 14, 13, 12 and 9. If we gave the preparation time to recover from the effects of the subminimal stimuli, stimulation of the bladder at 10 cm. would cause marked skeletal reflexes. It was plain that in the strychninized frog subminimal stimuli have depressing action on the cord.

CONCLUSIONS

1. Mechanical or electrical stimulation of the lungs, the gall bladder, the heart, the urinary bladder and the entire gastro-intestinal tract induces skeletal reflexes both in decerebrated and in purely spinal preparations.

2. These visceral skeletal reflexes, at least as regards the extremities, are essentially of the defensive or escape type.

3. In the frog general ether anesthesia abolishes the viscero-skeletal reflexes before the complete suppression of the purely skeletal reflexes.

4. Strychnine in doses sufficient to induce violent spasms does not appreciably alter the threshold for the viscero-skeletal reflexes, but in strychninized preparations the skeletal responses from the viscera are augmented (strychnine spasm). Strychnine also greatly increases the fatiguability of the viscero-skeletal reflexes, and it is possible to induce the fatigue in the absence of all skeletal effects by repeated stimulation of the viscera with subminimal stimuli.

5. In the turtle the viscero-skeletal reflexes are decreased or inhibited by the brain. The apparent absence of this phenomenon in the frog is probably due to a greater degree of spinal shock in this animal.

6. The purely spinal character of the viscero-skeletal response in our preparations indicates the possibility of forced movements initiated from the visceral afferents, but it does not eliminate a synchronous component of pain in animals with the brain intact.

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AN ELECTROCARDIOGRAM AND AN ELECTROMYOGRAM IN AN ELEPHANT

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Since Waller discovered the possibility of recording the electrocardiogram by merely applying electrodes to the skin and connecting them with a suitable recording instrument, this procedure has been applied to a considerable variety of animals; dog (1), (2), dog embryo (3), horse (4), (5), bird (6), tortoise (7), frog (8), goldfish (7) and eel (9), (10), (11). Electromyograms obtained from voluntary contraction of skeletal muscles in the intact subject, so far as we are aware, have only been made in the case of human beings.

In June, 1919, an unusual opportunity presented itself to obtain an electrocardiogram and an electromyogram from an elephant. On this occasion we learned that through the kindness of the Barnum & Bailey and Ringling Brothers Circus a number of exhibits were being presented to the patients in the Children's Hospital next door to the Harvard Medical School. Two elephants were awaiting their turn just outside our laboratory. Through the kindness of the officials of the Company we were permitted to obtain the desired records from one of these animals before he was taken away from the premises. Time did not suffice for the preparation of adequate apparatus for doing justice to the material at hand. The only electrodes we had ready at our disposal were those made of glass funnels covered with bladders, described in a previous communication (14), designed for application to the human forearm. These, on account of their small surface, were ill-adapted to make a satisfactory contact with the body of an elephant, especially since the resistance of the hide is so great that an extra large surface of contact should properly be used to offset it. The records were made with the Cambridge String Galvanometer which has been used in this laboratory for some years. It was provided with a gilded quartz string of 20,000 ohms resistance. Wires were led out of the window to the ground where the elephant stood,

and connected with two of the funnel electrodes already mentioned. The prospect of deriving enough current through the thick dry skin of this animal to make good records, seemed rather poor. We selected the skin of the axillae for our points of contact, as being the softest and therefore probably having the best conductivity. In order to reduce the resistance of the skin as much as possible it was moistened with Ringer solution. One electrode was applied to each axilla; they were held in position by two men, each wearing a rubber glove to insulate the electrode from himself. As soon as we tried to record the electrocardiogram it was found necessary to remove the wire whereby the core of the Cambridge instrument is put to earth, since the animal, standing as he was on the ground, was himself electrically grounded, and a short-circuit resulted. As soon as this wire was removed the electrocardiogram was easily obtained.

Fig. 1. Electrocardiogram of elephant. Resistance of string, 20,000 ohms; tension 1 cm. = 2.5×10^{-7} amp. Time is shown in 0.01 second intervals.

Unfortunately the camera with which the record was made was designed for higher speed work than electrocardiograms; that is, for the reactions of nerves and skeletal muscles. It was consequently impossible to make the film travel slowly enough to make a well proportioned record. Two successive heart beats are shown in figure 1. The record appears greatly deformed as compared with ordinary electrocardiograms, for two reasons, one being the fact that the speed of the film was so much greater than that ordinarily used, the other the fact that the enormous resistance of the elephant's hide reduced the current passing through the instrument so much that the excursions of the string were extremely small. If proper leads could be obtained and the film slowed down to the usual speed there cords would undoubtedly resemble more closely the electrocardiograms obtained from other animals.

By looking obliquely at the electrocardiogram in figure 1 in such a way that it appears shortened horizontally, the true shape of the wave can be seen. Preceding the main excursion (ventricular), is a small

slow dicrotic excursion with its highest point (the second peak) about 0.3 second before the beginning of the ventricular excursion. This is presumably the auricular action current. Following the ventricular excursion, there is a slight excursion which is presumably to be identified with the "T-wave." These excursions were found by oblique inspection of the film to be present consistently throughout the record, but with slight variations from cycle to cycle. An attempt has been made to reproduce the curve as it would have appeared had the contacts with the animal's body been as good as is usually the case in clinical records. We measured the deflections as accurately as possible with a hand lens, and plotted a curve with the ordinates magnified by an amount which would make the major excursion appear in the same ratio to the horizontal distance representing a second as it does

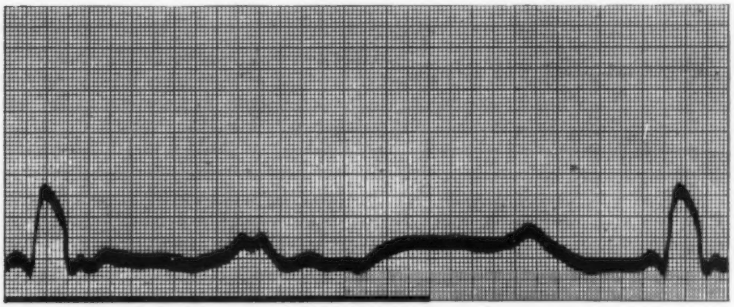

Fig. 2. Plotted curve of electrocardiogram with excursions magnified; see text. One second's duration shown below curve.

in the typical human electrocardiogram shown by Bayliss (13). The resulting curve is shown in figure 2; the possible error in making these measurements is large compared with the smaller excursions, but we believe the curve is a fair approximation.

The electrocardiogram shows an average heart rate of 41 per minute. The high speed of film made it easy to measure accurately the interval between successive ventricular action currents. In an uninterrupted record showing nine successive beats, the intervals were measured and found to be (in seconds) as follows; 1.49, 1.48, 1.43, 1.50, 1.44, 1.53, 1.44, 1.40. There is here a difference of 9 per cent between the longest and the shortest. It is also interesting to note a distinct tendency to alternate between long and short intervals; but it is also evident that the alternation is not perfectly sustained.

In order to record the electromyogram, the muscles of the trunk (proboscis) were selected as the most favorable. It was found that if an electrode was pressed against the ventral surface of the trunk near its tip, the animal would contract the muscles in such a way as to grip the electrode as indicated in figure 3. The other electrode was placed on the forehead. With this arrangement electromyograms were

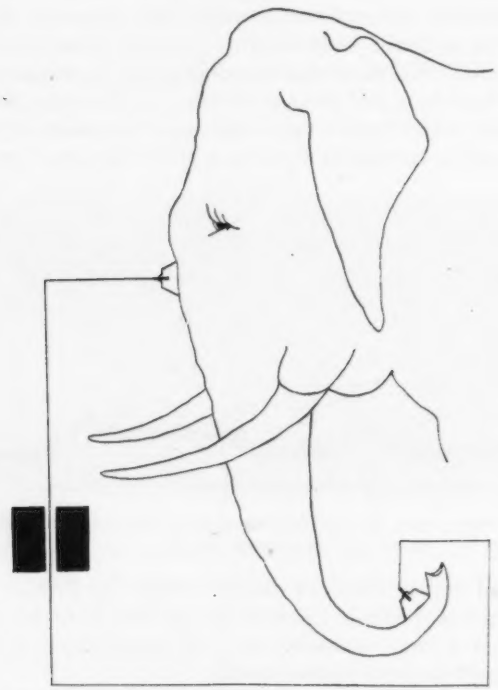

Fig. 3. Diagram showing the placing of electrodes in recording electromyogram.

recorded showing a sustained contraction and one of brief duration; both are shown in figure 4.

This electromyogram shows no marked difference from those obtained in human beings. Like the human electromyogram it shows periods of fairly regular action-current rhythm, interspersed with more irregular activity. The frequency of the main waves is about 48 per second, a

frequency agreeing closely with that commonly found in the muscles of the human forearm.

We wish to thank Miss A. Hopkins for her help in preparing the figures, especially in drawing figures 2 and 3.

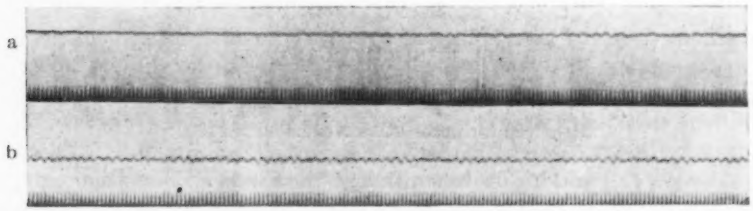

Fig. 4. Electromyogram of muscles in elephant's trunk in voluntary contraction; *a*, contraction of brief duration; *b*, part of prolonged contraction. String resistance and tension as in figure 1. Time in 0.01 second intervals.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

LVI. Gastric Secretion in Starvation

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Pawlow (1), working on dogs with a gastric fistula and esophagotomy, found that after the second day of starvation, the amount of gastric secretion on sham feeding decreased until at about the fifth day there was none, though the dogs were still lively and healthy. If the dogs were then given large amounts of water by fistula each day, the psychic secretion came back to normal, but decreased again until there was none on about the eighth or ninth day. If now 0.7 per cent NaCl was given instead of water, the secretion returned to normal. If NaCl was discontinued, the secretion decreased; if NaCl was given again, the secretion came back to normal. If on the days of starvation, when the dog would not eat, NaCl solution was given by fistula, and food was again presented 2 to 3 hours later, the dog would have a normal appetite and a normal amount of gastric secretion. The appetite is therefore influenced by the water and NaCl content of the blood.

Boldyreff (2), using dogs with intestinal and gastric fistulae, and one with a pancreatic fistula in addition, claims that in starvation longer than 24 hours, the stomach almost invariably shows periods of spontaneous gastric secretion, which last indefinitely and may become continuous. He does not give figures as to the amount secreted nor the degree of acidity but concludes that this secretion stops the peristaltic contractions of the stomach.

Carlson (3), studying the hunger contractions in prolonged hunger, says "the quantity or strength of hydrochloric acid in the stomach at any one time was not sufficient to produce acid inhibition in man, dog or rabbit." In a later experiment of 15 and 8 days starvation periods on man, reported in 1918, Carlson (4) found no significant increase or decrease in the continuous secretion of gastric juice, as compared with the control periods on the same individual. It is to be noted that dur-

ing the starvation periods the man drank an abundance of water (200 to 1800 cc.) daily. Carlson thinks that in Boldyreff's experiments, the stoppage of periodic gastric contractions after a fast of 3 or 4 days is due either to asthenia of the digestive tract as a result of the duodenal, biliary, pancreatic and gastric fistulae, or else to a lack of delicacy in the recording apparatus.

In connection with work on the intravenous injection of water as a stimulus to gastric secretion, it was thought that dilution of the blood might possibly explain the periodic secretion during starvation. Benedict (5) gives a resumé of the large amount of literature on the changes in the blood during starvation. The results are variable. Some writers claim a dilution, others a concentration of the blood. Such variations are often attributed to variations in water intake.

METHOD AND RESULTS

The starvation secretion was studied in four dogs, one with a Heidenhain pouch, one with a Pawlow pouch, and two with simple gastric fistulae. The juice was collected for 8 to 10 hours a day, and then the dog was allowed to drink all the water desired, and this amount was measured. In dogs with an isolated pouch, the juice was collected every hour; in the gastric fistula dogs collections were made every half-hour. Only the secretion rate per hour is given in the tables, which makes the changes from one collection period to the next less marked than they would be if given by half-hour periods. The juice was titrated against dimethylamidoazobenzol and phenolphthalein for free and total acidity respectively.

A dog with a Heidenhain pouch (no. 1) was starved at two different times for periods of 8 and 11 days respectively. The first time (table 1), the dog was given water to drink. In this case there was a gradual decrease in the hourly secretion of juice as the starvation continued, and there were periods of apparently spontaneous secretion, shown most definitely by the free acidity. In general the acidity was low, but this is to be expected in a dog with such a high threshold of stimulation. On only one day (the fourth) did the rate approach that of the normal digestion secretion. The same dog was starved a second time (table 2), and was given 0.9 per cent NaCl to drink for the first 7 days. The dog drank more NaCl solution than it had taken water. The amount of secretion did not decrease daily, nor were there any periods of free acidity, though there was a gradual, slight increase in

the total acidity. After the seventh day, the dog was given water to drink, and on the ninth day there was free acid in the juice.

A Pawlow pouch dog which was given 0.9 per cent NaCl to drink during a nine day starvation period (table 3) showed a continuous secretion without free acid for the first 4 days. After the fifth day, however, the free acidity remained almost constantly above 0.2 per cent. In this case as with dog 1, when given NaCl solution to drink, a secretion with free acid was late in starting.

TABLE 1

Dog 1, with a Heidenhain pouch. First starvation period. Body weight at the beginning of the experiment 9.0 kilos; at the end of the experiment 8.2 kilos

DAY	AMOUNT IN CUBIC CENTIMETERS PER HOUR										DRANK WATER
	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6	Hour 7	Hour 8	Hour 9	Hour 10	
											cc.
II	4.0	5.0	3.8	4.2	3.6	4.0	5.6	4.4	5.0	4.5	?
III	3.7	3.6	3.7	3.8	4.3	4.2	3.1	3.8	3.7		86
IV	1.6	2.5	2.1	3.1	3.0			3.3	3.3		76
V	1.6	2.1	2.3	2.8	2.7	3.0	2.4	3.0	2.6		80
VI	2.2	2.8	2.5	2.5	2.5	2.7	2.4	2.3	3.2		165
VII	1.2	1.8	2.0	2.4	2.4	2.8	1.7*	2.8	2.5		
VIII	1.3	1.7	2.6	2.6	2.3	2.3	2.8	2.1	2.1		

DAY	PER CENT FREE HCl										
II	–	–	0.02	0.02	0.03	–	–	0.02	0.03	–	
III	–	–	0.03	0.02	–	–	–	–	–		
IV	0.07	0.13	0.14	0.20	0.16			0.17	0.15		
V	–	0.03	0.05	0.07	0.05	0.05	0.05	0.04	0.05		
VI	–	–	–	–	–	0.02	0.02	–	–		
VII	–	0.05	0.05	0.03	0.01	–	–	0.02	0.08		
VIII	–	–	–	–	–	0.02	0.02	0.05	0.05		

* Given 500 cc. by stomach tube.

A dog with a simple gastric fistula (table 4) which was given water to drink, showed a periodicity in secretion beginning on the first day. The free acidity remained high, 0.2 to 0.4 per cent most of the time. In general there was a decrease both in acidity and the amount of juice toward the end of the experiment. At the end of the seventh day of observation the dog was very weak and would not eat. This may have been due to NaCl deficiency.

TABLE 2

Dog 1, with a Heidenhain pouch. Second starvation period. The dog was given 0.9 per cent NaCl to drink for the first 7 days and after that water. Body weight at the beginning of the experiment 10.1 kilos; at the end of the experiment 8.2 kilos

DAY	AMOUNT IN CUBIC CENTIMETERS PER HOUR									CUBIC CENTIMETERS OF 0.9 PER CENT NaCl OR H_2O DRANK
	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6	Hour 7	Hour 8	Hour 9	
I	4.0	5.3	4.2	7.2	?	7.2	7.0	4.0	4.3	—
II	2.9	4.5	3.2	3.0	5.7	2.4	3.0	4.2		275 NaCl
III	3.2	3.5	5.0	3.8	3.3	4.3	5.5	6.4	3.6	240 NaCl
IV	3.4	5.4	5.4	5.1	4.9	4.5	4.3	4.0		210 NaCl
V	Not collected									310 NaCl
VI	4.6	5.1	5.5	6.3	5.8	5.5	5.0	4.3	5.4	155 NaCl
VII	5.3	5.1	5.3	4.8	4.7	5.2	4.3			300 H_2O
VIII	5.0	5.2	5.4	4.8	5.2	5.5	4.8	5.2	5.2	110 H_2O
IX	2.6	3.0	3.6	3.5	2.8					140 H_2O
X	4.6	5.2	4.8	5.1						200 H_2O
XI	4.8	4.6	3.6	3.6	4.0					

DAY	ACIDITY OF JUICE, PER CENT HCl									
I	—	—	—	—	—	—	—	—	—	Free
	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	Total
II	—	—	—	—	—	—	—	—	—	Free
	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01		Total
III	—	—	—	—	—	—	—	—	—	Free
	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.02	Total
IV	—	—	—	—	—	—	—	—	—	Free
	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.01		Total
VI	—	—	—	—	—	—	—	—	—	Free
	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.03	Total
VII	—	—	—	—	—	—	—	—	—	Free
	0.03	0.03	0.03	0.03	0.03	0.03	0.04			Total
VIII	—	—	—	—	—	—	—	—	—	Free
	0.02	0.02	0.03	0.03	0.04	0.04	0.03	0.04	0.04	Total
IX	0.06	0.05	0.04	0.05	0.01					Free
	0.12	0.11	0.09	0.10	0.08					Total
X	—	—	—	—	—	—	—	—	—	Free
	0.03	0.03	0.03	0.03						Total
XI	—	—	—	—	—	—	—	—	—	Free
	0.03	0.02	0.04	0.06	0.05					Total

TABLE 3

Dog 12, with a Pawlow pouch. Dog was given 0.9 per cent NaCl to drink. Body weight at the beginning of the experiment 5.5 kilos; at the end of the experiment, 4.8 kilos

DAY	AMOUNT IN CUBIC CENTIMETERS PER HOUR									DRANK CUBIC CENTIMETERS OF 0.9 PER CENT NaCl
	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6	Hour 7	Hour 8	Hour 9	
I	1.2	2.4	1.9	2.9	2.1	2.3	2.2	2.6	2.2	85
II	2.0	2.2	2.3	2.2	2.4	2.4	2.1	1.7		95
III	2.2	2.4	2.3	2.2	2.6	2.1	2.2	2.1		150
IV	2.3	1.8	2.4	2.7	2.0	2.1	2.8	2.4		180
V				Juice not collected						150
VI	1.7	2.9	2.9	3.2	4.6	4.6	4.5	4.4	4.0	160
VII	1.3	1.7	1.6	1.1	1.7	1.7	1.8			140
VIII	1.5	1.2	1.2	1.8	2.1	2.1	1.5	2.1	2.6	95
IX	1.3	1.5	2.0	2.5*	7.0					

DAY	PER CENT FREE HCl									
I	–	–	–	–	–	–	–	–	–	
II	–	–	–	–	–	–	–	–		
III	–	–	–	–	–	–	–	–		
IV	–	–	–	–	–	–	–	–		
V										
VI	0.03	0.09	0.18	0.19	0.21	0.26	0.26	0.28	0.25	
VII	0.21	0.28	0.28	0.28	0.25	0.24	0.26			
VIII	0.20	0.21	0.23	0.29	0.31	0.31	0.31	0.32	0.34	
IX	0.21	0.23	0.29	0.32*	0.35	(last half hour 0.41)				

* Fed 50 grams fresh steak.

A dog from which all the gastric juice is collected for 8 to 10 hours a day loses a considerable amount of chloride from the body. Calculating the chlorine on the basis of the titrations for free HCl, the chlorine lost from the body in the gastric juice in this case was:

	DAY						TOTAL
	2	3	4	5	6	7	
Cl in grams.....	0.45	0.23	0.15	0.34	0.10	0.01	1.20

According to figures given by Mathews (6), this would be equal to the total amount of chloride in the blood at the beginning of the experiment, and about one-fourth the total amount of chlorine of the body.

Another dog with a simple gastric fistula (table 5), which was given physiological saline solution to drink, also showed a periodicity of secretion, the acidity often running over 0.45 per cent free HCl in samples taken for shorter periods than an hour, without the regurgitation of bile. (Such a high degree of acidity was followed soon by a regurgitation of bile.) There was slight if any decrease in the hourly amount of secretion toward the end of the starvation period; certainly the decrease was not as marked as in the dogs which were given only water to drink. This dog did not lose its appetite since on the sixth

TABLE 4

Dog 11, with a simple gastric fistula. Dog was given water to drink. Body weight at the beginning of the experiment 6.1 kilos; at the end of the experiment, 5.7 kilos

* indicates that bile was present in the juice collected

DAY	AMOUNT IN CUBIC CENTIMETERS PER HOUR										DRANK IN CUBIC CENTIMETERS OF WATER
	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6	Hour 7	Hour 8	Hour 9	Hour 10	
II	11	17*	20	23	11	33*	9	10		15*	
III	8	12	9	10	7*	8	7	13	10		294
IV	13	16	14	3	2	3		3	2		180
V	14	21	26	23*	5	8	5	3			160
VI	7*	8	8	9	3	5	7	5	4		100
VII	8	3	2	4	3	2					

DAY	PER CENT FREE HCl										
II	0.31	0.25	0.35	0.29	0.26	0.22	0.28	0.23		0.29	
III	0.23	0.35	0.26	0.27	0.29	0.21	0.25	0.32	0.29		
IV	0.29	0.32	0.24	0.33	0.31	0.28	0.26	0.17			
V	0.25	0.36	0.36	0.25	0.20	0.23	0.15	0.18			
VI	0.10	0.25	0.27	0.22	0.12	0.17	0.16	0.12	0.08		
VII	–	0.01	0.02	0.04	0.01	–					

and seventh days there was evidence in the juice of its having eaten its own fecal material, and the dog ate well at the end of the starvation period.

In starvation, therefore, there is a continuous secretion, which shows periods of more rapid secretion. That this is not an artifact due to imperfect drainage in the gastric fistula dogs is indicated by the fact that the acidity of the juice rather closely parallels the amount of juice. Furthermore, it occurs in dogs with an isolated pouch, in which the pouch is filled by the drainage tube. In gastric fistula dogs, this periodicity started on the first day; in Pawlow and Heidenhain pouch

dogs it may not start for several days. In these starvation experiments, in which no liquid had been given for 10 to 14 hours before the experiment, periodicity in gastric secretion can hardly be due to variations in blood dilution.

In dog 10 (table 5) the amount of juice and acid secreted at several different times in the experiment would presumably have been

TABLE 5

Dog 10, with a simple gastric fistula. The dog was given 0.9 per cent NaCl to drink. Body weight at the beginning of the experiment 7.7 kilos; at the end of the experiment 6.2 kilos

DAY	AMOUNT IN CUBIC CENTIMETERS PER HOUR									DRANK CUBIC CENTIMETERS OF 0.9 PER CENT NaCl
	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6	Hour 7	Hour 8	Hour 9	
I	63	28	9	8	10	9	5	10	36	195
II	18	25	20	30	16	15	7	14		200
III	13	7	54	14	14	12	11	12	1	325
IV	1	27	lost	6	lost	30	18	23		450
V				Juice not collected						325
VI	37	25	31	6	1	30	6	15	14	80
VII	115	40	13	16	4	7	14	(11)		205
VIII	17	14	4	6	15	13	20			250
IX	21	08								

DAY	PER CENT HCl (FREE)									
I	0.39	0.40	0.31	0.26	0.19	0.16	0.06	0.22	0.27	
II	0.34	0.40	0.41	0.37	0.34	0.35	0.17	0.22		
III	0.36	0.20	0.44	0.39	0.32	0.40	0.32	0.44	0.33	
IV	0.12	0.40		0.28	—	0.23	0.24	0.20		
V										
VI	0.33	0.30	0.38	0.26	0.11	0.36	0.35	0.31	0.33	
VII	0.20	0.39	0.45	0.31	0.37	0.16	0.31	0.29	0.38	
VIII	0.35	0.40	0.34	0.30	0.36	0.35	0.38	0.38		
IX	0.38	0.34								

enough to inhibit hunger contractions. However, there was no tendency for a more constant high secretion on the second or third or succeeding days of starvation than on the first, as reported by Boldyreff.

SUMMARY

1. In starvation, there is a continuous gastric secretion which shows apparently spontaneous periods of more rapid secretion.

2. If the dog is given only water to drink during a starvation period, the amount of gastric secretion decreases in general day by day. If the dog is given 0.9 per cent NaCl to drink, the amount of gastric secretion remains about the same at the end as at the beginning of the starvation period.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

LVII. The Response of the Stomach Glands to Gastrin before and shortly after Birth

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The fact that "gastrin" as prepared by Koch, Keeton and Luckhardt (1) produces invariably an almost quantitative response of the gastric glands in adult animals, was made use of to determine the earliest evidence of capacity of the gastric glands to secrete hydrochloric acid during intra-uterine life.

Hammarsten (2) in 1875 stated that the gastric secretion of newborn dogs and cats contains acid strong enough to coagulate milk. Gmelin (3) claimed that there is no HCl in the stomach of pups at birth, but only lactic acid, and that HCl does not appear until the eighteenth to twenty-fifth days. Cohnheim and Soetbeer (4), doubting Gmelin's results, performed esophagotomy on pups in order to secure juice unmixed with food. They collected the gastric secretion by means of a Nelaton catheter during the "sham feeding" of the pups and found free HCl as early as the first day, and the fourth day after birth, using Günzberg's reagent as the test for free HCl. Gmelin (5) repeated these experiments by the same method except that he did not wash out the stomach with water at the beginning of the experiment. He held to his former conclusions, and claimed that the kind of catheter used by Cohnheim and Soetbeer, when washed with water, gave a distinct though weak acid reaction. Hess (6) found free HCl in the stomachs of infants before the first feeding, the free acidity varying from 15 to 120 clinical units with an average of about 40. He noted a continuous acid secretion in tests lasting for one to two hours.

METHODS

In these experiments the gastric juice was collected either through a simple gastric fistula or by means of a stomach tube. The stomach

was stimulated to secretory activity by the subcutaneous or intramuscular injection of gastrin.[1] Thus the juice or gastric content was obtained free from food. In pups and kittens the first tests were made after making a gastric fistula, but it was found that the stomach tube was just as efficient, without involving the necessity of an aseptic operation. In rabbits and guinea pigs the passing of the stomach tube was not successful, so the few experiments on these animals were performed after having made gastric fistulae. The stomach tube used was very small-sized, fairly stiff rubber tubing. The stomach contents, usually only a few drops, were put on a white tile and tested with dimethylamidoazobenzol. In case this showed free acid a further test was made with Günzberg's reagent. Litmus paper was used in some cases in which there was not enough acid to affect the other indicators. The presence of mucus in the contents hindered these tests, especially the Günzberg. In some cases rinsing the drop of gastric mucus with distilled water and testing this wash water gave an acid reaction, when the tests on the mucus and water beforehand had been negative.

In some of the animals the stomach was not emptied at the beginning of the experiment.

The young were taken from the uterus by operation or from the mother immediately after birth, some of the young were injected with gastrin and others left as a control. In some cases the young were killed, about an hour after the gastrin injection, the ends of the stomach tied, and the contents secured as nearly quantitatively as possible. Chlorine determinations were made on these by the McClean-Van Slyke (7) method.

RESULTS

1. Pups. Acid was found to be first secreted at about the time of birth. The injection of gastrin in pups taken from the uterus 1 to 3 days before birth (table 1) caused an increase in the amount of stomach contents (gastric secretion, saliva, or both), and in the amount of chlorine in the stomach, but no change in the percentage of chlorine. There was an increase in the gastric acidity in the pups near term (D 7, 8 and 10), but not enough to give a positive test with Günzberg's reagent, when the secretion was diluted with meconium. There was evidence in one pup (D 8) that free acid can be secreted before birth;

[1] The gastrin used in these experiments was made by Dr. F. C. Koch.

in others (table 2) it appears only on the first or second day after birth. Pups 40, 41 and 43 were from the same litter and are of special interest because more than one test was made on each animal. Some of the tests were negative and others doubtfully positive on the first day; the tests were more definite on the second day, and distinctly positive on the third day.

2. Kittens. The injection of gastrin at birth or a few days before birth caused an increase in the amount of stomach contents, and in the amount of chlorine, but not in the percentage of chlorine in the

TABLE 1

Pups before birth

D 7, 8, 10 and 11 were taken from the uterus of a 15 kilo bitch, using local anesthesia. They probably would have been born within 24 hours. D 32, 33 and 35 were taken from a 12 kilo bitch, probably 2 to 3 days before birth. The reaction in D 8 was not acid to Günzberg's reagent; in D 10 it was not acid to dimethylamidoazobenzol.

Stomach contents secured on killing animals about 1 hour after gastrin injection.

ANIMAL NUMBER	WEIGHT	GASTRIN INJECTED	REACTION	STOMACH CONTENTS		
				Amount	Chlorine	
	grams	*cc.*		*grams*	*per cent*	*mgm.*
D 7	245	0.4	Neutral (litmus)	0.88	0.42	3.68
D 8	250	0.4	Acid (dimethyl.)	0.42	1.52	6.63
D 10	237	0.4	Acid (Congo R.)	0.11	1.47	1.56
D 11 (control)	218		Neutral (litmus)	0.08	1.42	1.13
D 35	205	1.5	Neutral (litmus)	0.10		
D 33	195		Neutral (litmus)	0.05		
D 32	202		Neutral (litmus)	0.05		

stomach contents (table 3). In kittens only a little earlier in development (nos. 15 to 18) gastrin caused no increase in stomach contents. If the stomach was emptied before the gastrin injection (table 4), free acid was always found on the first day of extra-uterine life. In the case of one kitten (no. 48) taken from the uterus 1 to 3 days before birth, the stomach contents after the injection of gastrin gave a positive test with Günzberg's reagent.

3. Guinea pigs. (table 4). Not only was acid found after gastric injection, but in two animals taken from the uterus (nos. 17 and 23)

the stomach contents were found to contain free acid when the stomachs were opened in making the fistulae. In other young from the same mother, however, the stomach contents were not acid. The guinea pig is relatively mature at the time of birth.

TABLE 2

Pups after birth

Showing stronger acid secretion. The exact time of birth of the pups was usually not known, but in no case was the animal older than the age given here; + means that the reaction was acid; ++ means strongly acid; and ± means a doubtful reaction. The gastric juice was collected by stomach tube or fistula.

NUMBER OF ANIMAL	AGE	BODY WEIGHT	LITMUS	DIMETHYL-AMIDOAZO-BENZOL	GÜNZBERG'S
		grams			
3	5 days		+		−
3	7½ days			++	++
13	12 hours	234	+		
14	12 hours	150	+	−	
21	24 hours	215		++	
26	18 hours	158		+	
27	24 hours	180		+	
28	1½ days	147	+	−	
20	12 hours	220		++	+
22	36 hours	202		++	++
19	2½ days	264		++	++
18	7½ days	165		++	++
40	24 hours	187	+	−	
40	3 days	245		++	+
41	24 hours	177		+	
41	1½ days?			++	+
43	2 days				±
43	3 days	205			+

4. Rabbits (table 4). The earliest satisfactory test was made on an animal on the second day after birth, and this test was positive for free HCl. Since the rabbit is fairly well developed at birth, probably a longer series of experiments would show that acid can be secreted during intra-uterine life.

SUMMARY

1. In guinea pigs there may be a "spontaneous" secretion of acid gastric juice during late intra-uterine life.

TABLE 3

Kittens, at and before birth

Stomach contents were secured on killing the kittens about one hour after gastrin injection. There is no reaction in litter D (1 week before birth), but an increase in stomach contents in litters A and B (at birth) and also an increase in acidity in litter C (2 to 3 days before birth).

ANIMAL NUMBER	LITTER	AGE	AVERAGE WEIGHT	GASTRIN INJECTION	STOMACH CONTENTS	
					Reaction	Amount
						grams
8, 9, 11, 14	A and B	At birth		+		1.31
10, 13	A and B	At birth		−		0.01
43, 44	C	2 to 3 days before birth	83	+	+ tr. dimethyl.	0.80
42	C	2 to 3 days before birth	79	−	Neutral to litmus	0.07
15, 16, 18	D	1 week before birth	67	+		0.31
17	D	1 week before birth	70	−		0.39

TABLE 4

Kittens, rabbits and guinea pigs

Showing early appearance of HCl. Age given is the time *after* birth unless otherwise stated.

	ANIMAL NUMBER	AGE	REACTION OF STOMACH CONTENTS	
			DIMETHYL-AMIDOAZO-BENZOL	GÜNZBERG'S
Kittens	5	24 hours		++
	6	24 hours		++
	1	36 hours		++
	23	36 hours		++
	48	1 to 3 days before birth		++
	8	3 hours	+	
	10	3 hours	+	
Rabbits	8	2 days		++
	1	4½ days		++
Guinea pigs	4, 5, 6	36 hours		++
	17	1 to 6 days before birth	++	++
	23	1 to 6 days before birth	++	++

2. In dogs and cats there appears to be no spontaneous gastric juice secretion in utero, but there is some secretory response to gastrin when the fetuses are within a few days of term. Fetuses of the same litter show individual variations in capacity to respond to gastrin.

3. In the cat and the dog the gastric glands do not appear to be fully differentiated even at birth, as the secretory response to the gastrin injections increases with age, at least for the first few days.

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THE DUODENAL SEGMENT OF THE ALBINO RAT AS A STANDARD REAGENT FOR THE STUDY OF TISSUE EXTRACTS AND OTHER SUBSTANCES

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INTRODUCTION

In a previous paper (1) we described certain factors which prevent a uniform and consistent type of response by the isolated duodenal segment of the albino rat to stimulation by 0.1 M. sodium carbonate. That report dealt solely with the qualitative nature of the response elicited. The present paper is concerned with the study of the quantitative reaction of such a segment in order to determine its availability as a standard reagent for the study of tissue extracts and reactions caused by various other substances.

That some mechanism of the isolated segment of unstriated muscle tissue responds to the application of one substance or another by alterations in rhythm, amplitude and tone of contraction has been adequately demonstrated by the studies of Magnus (2), (3), Roth (4), Alvarez (5), Fawcett and his collaborators (6), Collip (7) and others; consequently an intensive review of the literature is unnecessary.

In the attempts to use unstriated muscle preparations for the standardization of various pharmacological substances the uteri from one animal or another have been mainly employed. Kehrer (8), using such material for the assay of ergot and its derivatives, probably was the first to record studies of a quantitative nature. Roth (4) and more recently Spaeth (9) have contributed largely to the refinement of the method by the use of the isolated uterus of the virgin guinea pig as a test-organ for the determination of the strength of pituitary extracts. When, however, we consider the studies made of the quantitative response of the isolated intestinal segment, it is found that satisfactory data are lacking.

In any quantitative study of this type it is essential that one should have some standard stimulating substance to which the response of the segment will be quantitatively uniform within prescribed limits. The prime requisites of such a substance are purity, definiteness, solubility, diffusibility, non-reaction with the fluid in which the segment is suspended, rapidity of action, lack of toxicity and non-permanency of effect. Also it should not be a compound foreign to the reacting tissue. There are found scattered through the literature several significant observations relative to the qualitative action on the intestine of various sodium salts of weak acids. Salant and Schwartze (10) and Salant, Mitchell and Schwartze (11) found that sodium citrate, succinate, malate and tartrate stimulate the intestine, the results depending, however, upon the strength of the solution and the part of the intestine studied. Starkenstein (12) noted that 0.2 N. sodium pyrophosphate caused contraction of the isolated rabbit gut, and Alvarez (13) observed in his study of the action of drugs on intestinal rhythm, that sodium bicarbonate apparently has no effect on the rate of contraction but does have a definite effect on the tone and on the amplitude. Several observers have reported that the response to these stimulating substances is usually weaker the weaker the solution used, all of course within certain limits. With these facts in mind and because I had previously found that sodium carbonate called forth a uniform and consistent type of response when applied to the isolated duodenal segment of the adult, unexcited, male, albino rat, 17 to 18 hours after the last feeding, this compound was chosen as likely to meet the requirements mentioned above.

The choice of animal from which the test material was obtained was obviously directed by the fact that at The Wistar Institute the albino rat is the experimental animal commonly used, and in addition by previous experiences with cats and guinea pigs which had shown me that these animals are decidedly inferior to the albino rat for such studies because of their inherent generic, physiological dissimilarity and instability. Moreover, as has been shown (1), in order that a consistent type of response may be had it is essential to use male animals of a certain known age, normal size and emotional history. All of these conditions can be well met by the albino rat.

Apparatus. There have been various forms of apparatus designed for investigations of this sort, that of Roth (4) being in principle the one commonly used in this country, while that described by Uhlemann and Abelin (14) in 1920 is probably the most recent modification.

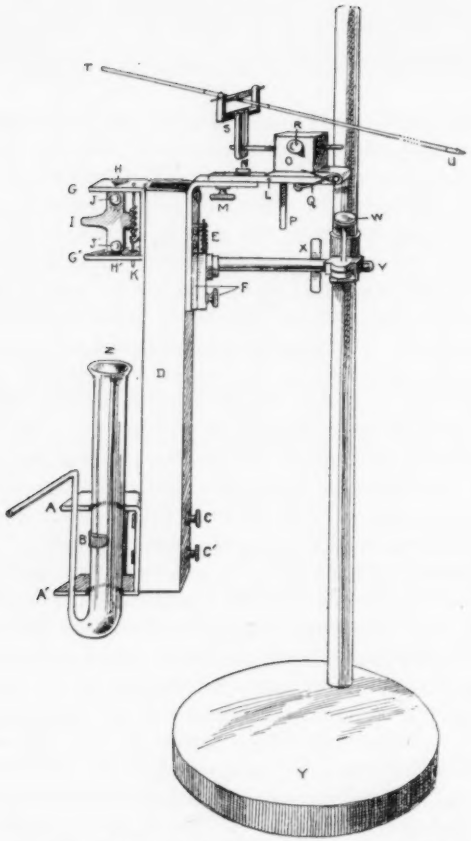

Fig. 1. The Greenman lever- and cell-support: *A–A′*, Cell-support, 6.2 × 3 cm. *B*, Hook for holding cell firmly in support. *C–C′*, Thumb-screws playing in slots allowing of adjustment of cell support to different angles from the vertical or horizontal to the shank. *D*, Shank, hollow, rectangular; 18 cm. × 1.5 cm. × 1.5 cm. *E*, Spring to hinge by which lever point is approximated to or removed from the drum of kymograph. *F*, Thumb-screw regulating play of spring *E*. *G–G′*, Support for the glass tube to which the segment is attached. *H–H′*, Triangular openings through which the glass tube is inserted or removed. *I*, Spring clamp to hold glass tube firmly in place; also allows its adjustment to required depth in the cell. *J–J′*, Rubber discs against which the tube is held. *K*, Spring acting on clamp *I*. *L*, Lever carrier. *M*, Thumb-screw allowing horizontal adjustment of lever carrier. *N*, Set-screw for fixing horizontal adjustment of lever carrier. *O*, Lever standard support. *P*, Thumb-screw for raising or lowering the lever standard support. *Q*, Spring for imparting stability to action of Q. *R*, Thumb-screw allowing rotary or horizontal adjustment of lever standard. *S*, Lever support. *T*, Lever. *U*, Writing point. *W*, Thumb-screw for rotary or horizontal adjustment of apparatus as a whole. *X*, Thumb-screw for clamping apparatus to upright on weighted stand at any desired height. *Y*, Weighted stand for carrying the apparatus. *Z*, The glass cell with its side arm.

The device used in these investigations, however, presents certain advantages in compactness and accessibility that deserve a brief description. Figure 1 is a drawing of the apparatus designed and made by Doctor Greenman of the Wistar Institute. It is constructed of brass and its purpose is to combine a lever-carrying mechanism and a support for a glass cell in which the segment is suspended into a single piece carrying all the essential adjustments. The description of the various parts is given in the legend accompanying the drawing. The glass cell, *Z*, shown may be of two sizes. The smaller is 1 cm. in diameter, 10 cm. in length and has a side-arm made of 4 mm. glass-tubing, the vertical portion of which is 8 cm. and the horizontal 6 cm.; when full it holds a little over 8 cc. of solution. The larger cell holds about 15 cc. and is proportionately made. The piece of glass-tubing, *J*, through which the oxygen supply to the segment in the cell is maintained is also of 4 mm. tubing, and is 22 cm. in length to the top of the bend. The end dipping into the solution is fused and contains a small hole for the escape of the oxygen into the cell. A bit of platinum wire is fused into the tip and is bent into a loop for the attachment by a silk thread from one end of the segment. Figure 2 is a drawing of the whole apparatus as set up for use and is adequately explained by the legend. Special note should be made of the combined heating and thermo-regulating device, *C*, made by the C. S. & E. Co. I have found during some 6 months' use that this apparatus is most satisfactory in that it maintains 3 liters of water for hours at a time at any desired temperature between 37 and 39°C. with less than one-tenth of a degree variation. Beyond this range I have not tested the mechanism. It will also be noted that most of the constituent parts of the apparatus are usually to be found in the laboratory and that it is inexpensive.

Method. About one-half hour before the segment is prepared, 200 cc. of freshly made Tyrode's solution are put in the Erlenmeyer flask, *D*, which is immersed in the water-bath the contents of which are maintained at 39° throughout the experiment, and a small stream of oxygen is bubbled through to insure saturation. Some 5 minutes before the segment is prepared the cell (small) is washed twice with Tyrode's, filling the cell each time and sucking the contents into the waste-receiving-flask, *E*, and finally 4 cc. of the solution are put into the cell and the oxygen stream directed through it by means of the glass tube which is lowered into the cell. The oxygen flow is regulated to give about 40 bubbles a minute since marked variations in gas supply may modify the response of the segment, as was shown by Alvarez (14).

An unexcited male rat of 100 days in age or over is then lightly anesthetized with ether and killed by crushing the cord in the cervical region with bone-forceps. Occasionally this is followed by violent tetanic contractions depending upon the degree of anesthesia. An extensive longitudinal incision is immediately made through the abdominal wall, care being taken not to cut into the liver, the stomach is drawn down with forceps and the duodenum exposed and recognized

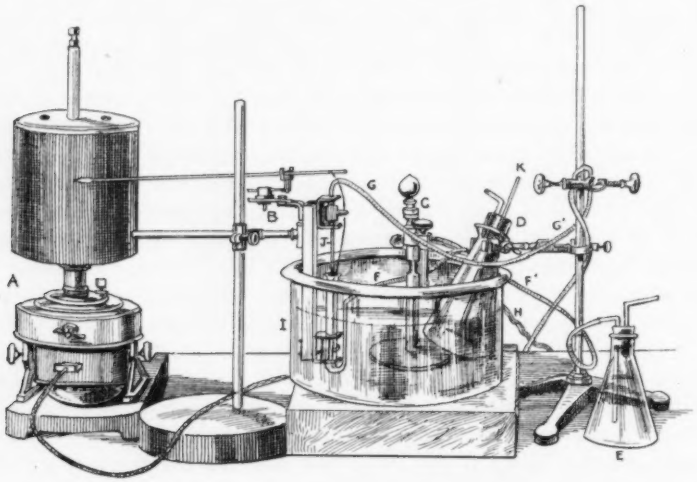

Fig. 2. The apparatus as set up for use: *A*, Electric kymograph. *B*, Stand and Greenman lever carrier. *C*, Heating and thermo-regulating unit. *D*, Flask containing oxygenated Tyrode's. *E*, Flask connected by tube.—*F*–*F'*, for sucking out contents of cell. *G*–*G'*, Rubber tubing for oxygen supply. *H*, Wires from light socket to heating device. *I*, Round museum jar of about 5 liters capacity. *J*, Glass tube for segment attachment and oxygen supply to cell contents. *K*, Tube for oxygenating Tyrode's solution in Erlenmeyer flask.

as the pinkish bit of intestine next to the stomach. The gastric end of the duodenum is then gently grasped with the forceps, severed from its gastric attachment and rapidly but gently and without undue tension stripped downwards by the scissors from its mesenteric attachments and the pancreas for about 3 cm. The segment is then cut from the distal portion of the intestine and immediately put into 5 to 10 cc. of Tyrode's solution contained in a glass dish floating in the water-bath. The glass tube in the cell is then raised so that the end in which the

platinum loop is fused is easily accessible, the segment is cut into two approximately equal parts, and silk threads are tied into the ends of that portion of the segment which was nearer the stomach. This piece is then attached to the platinum loop by one of the threads and immediately lowered without tension into the cell. The thread tied to the free end of the segment is now fastened to the lever, *T*, in such a fashion that the lever is approximately horizontal, and any deviation from the horizontal is adjusted by the thumb-screws, *M*, *P* and *R*. A segment so prepared is at once active and contracts rhythmically. In order to allow it to recover from the shock of handling I always permit the segment to trace its record for 10 minutes. Under these conditions the base-line soon becomes uniform and the contractions are regular in rate and amplitude. Any segments showing marked irregularities during this preliminary period should be discarded as unfit for quantitative work. After a preliminary tracing lasting 1 or 2 minutes the desired amount of 0.1 M. sodium carbonate is added by a graduated pipette to the liquid surrounding the segment in the cell which, it will be remembered, has a volume of 4 cc. The segment begins to shorten within a few seconds and continues so to do until it has reached its maximum contraction for the amount of carbonate used. The termination of this shortening under any given stimulus is determined by the fact that the new level of contractions becomes parallel to the original base line. When this has occurred the solution is sucked from the cell and the segment and cell are washed two or three times with oxygenated Tyrode's solution at 39°. Four cubic centimeters of the Tyrode's are then put into the cell. Within a minute or two, if not before, the segment has relaxed to the original base-line, or the initial condition of tone. One complete revolution of the drum is allowed for recovery from the test and the procedure is then repeated.

Results. Using the method as described, a study was made of the quantitative variability of the response of a given segment to a given amount of carbonate solution, using as a measure the height in millimeters of the maximum rise in level of contractions as traced by the writing point of the lever from the base-line originally recorded. In measuring this rise the distance between the two base lines formed by the terminations of the relaxation phase of the rhythmical contractions at the two levels was taken.

It was evident from the beginning that the first response to the carbonate stimulation is not quantitatively exact with respect to succeeding stimulations in most of the cases. This is probably due to a lack of

adjustment in the responsive mechanism. This is set in order by the first stimulation. It was also plain that as the experiments were conducted the response to stimulation after the fifth application of carbonate tended to be also quantitatively abnormal; at times showing evidences of what might be considered fatigue and at times giving evidence of hyperirritability. Consequently only the values obtained on the second, third, fourth and fifth applications of carbonate were used in calculating the degree of variability. A certain percentage of exceptions occurred, which are noted later. In addition there occasionally, but rarely, occurs in the series an abnormal reaction that is at present inexplicable. However, by and large, very satisfactory agreements are obtained between the successive shortenings of the segment from the second to the fifth stimulation inclusive. The accompanying tracing shows the points mentioned (chart 1).

For the material used in the calculations, the results of which are reported, measurements were made of the effect of 0.1, 0.2, 0.25, 0.3, 0.4 and 0.5 cc. of 0.1 M. sodium carbonate on the isolated duodenal segments when suspended in 4 cc. of oxygenated Tyrode's solution at 38 or 39°C. These strengths were used in order to obtain some notion of what amount of stimulus is best suited for general work. As a general thing 0.1 cc. tends to be too weak; while 0.4 and 0.5 cc. are apt to be too strong, causing a maximum contraction which obviously cannot be used as a criterion for relative measurements. Three-tenths or 0.25 cc. give quite uniformly consistent results in the dilution noted. To facilitate calculation the latter amount has been adopted as the standard quantity of stimulating substance.

In this study a total of thirty-seven segments was used, out of which seven were discarded because of marked initial irregularities in rhythm and tone. On the remaining thirty segments 196 observations were made of the response to sodium carbonate stimulation; of these responses 30 were not included in the calculation because they were first responses; similarly 53 were terminal results after the first 5 and were regularly discarded. This left as a residuum 113 observations. Eight of these were discarded because of irregularities of the response due to obvious experimental errors; catching of the thread and variations in temperature or in oxygen supply. Hence we have a total of 91 discards, which subtracted from the 196 observations gives 105 that were used in the calculations. Of these 105 observations 8 were included which had been obtained after the fifth stimulation, for in these cases the segments seemed to be quantitatively active beyond the fifth stimulation.

When the per cent deviation from the mean of each set of observations on the individual segments was calculated it was found that 90 per cent of the series gave values within a limit of variability of 10 per cent; while over two-thirds of these were within the 5 per cent limit. The data are represented in chart 2 and are seen to closely approximate

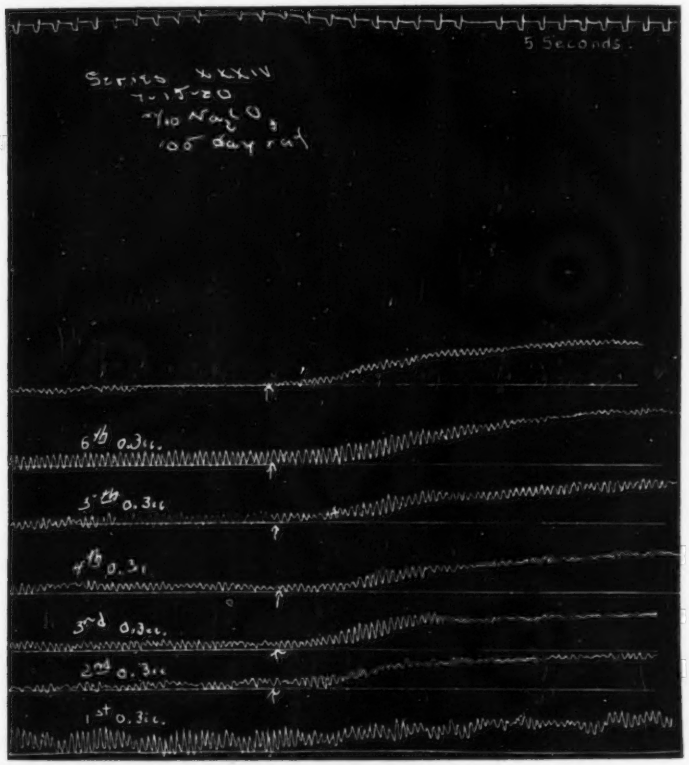

Chart 1. Tracings of quantitative response of duodenal segment to unit amounts of 0.1 M. sodium carbonate solution.

a probability curve in their contour. They indicate that for purposes of investigation it is possible to obtain an isolated intestinal segment, the response of which, by shortening to unit amounts of 0.1 M. sodium carbonate is generally quantitative within moderate limits. Such a segment can be used as a standardized test organ or reagent.

The procedure to be employed in the preparation of such a standardized segment is the same as that described for obtaining the above results. Twenty-five hundredths of a cubic centimeter of 0.1 M. sodium carbonate is used as the standard stimulus and three records of its effect are recorded, the first of which is always discarded. The mean of the second and third readings is used to determine the degree of contraction induced. If the per cent deviation from the mean

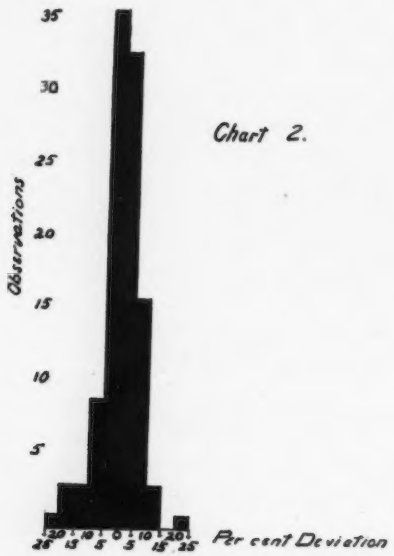

Chart 2. The distribution of the per cent deviation from the mean of the individual series of observations.

does not lie within the 5 per cent limit of variability the results are looked on with suspicion. The mean so obtained is used to compare with the height of contraction induced by any other substance. This permits the determination of an index of the relative effect of the solutions tested in terms of standard sodium carbonate effect. An effort should be made, however, to so adjust the strength of the unknown solution that the contraction caused by it is nearly the same as that caused by the carbonate, since there is, in most segments, only a limited

range within which alterations in the strength of the stimulant are quantitatively related in equality.

Applications. This method can be applied to a large variety of investigations. At present, in this laboratory, a study is about completed of the relative amounts of contraction induced in the duodenal segment by extracts of the thyroid gland in male and female rats at different ages. Similar studies of other tissues and their relative content of this principle as qualitatively studied by Stern and Rothlin (15) and Collip (16) are possible. Its use in the determination of the relative strength of various pharmacological preparations is also evident. Studies now in progress to determine the stimulating constituent of the sodium carbonate and its relation to the mechanism of the neuro-muscular control of the intestine demonstrate that the method can be applied to different types of investigation.

SUMMARY AND CONCLUSION

An apparatus and method are described by which the isolated duodenal segment of the albino rat can be so prepared and standardized as to be available for quantitative use as a test-organ in a wide range of biochemical, physiological and pharmacological investigations.

It was found that such a standardized segment will respond to stimulation by 0.1 M. sodium carbonate with a nearly uniform amount of contraction. In 66 per cent of the observations there was a deviation of plus or minus 5 per cent, and in 90 per cent of the observations the deviation was plus or minus 10 per cent. This latter is a generally satisfactory value for physiological work.

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THE INTESTINAL MECHANISM PRIMARILY STIMULATED BY SODIUM CARBONATE (ALBINO RAT)

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It has been shown in two previous communications (1), (2) that the isolated duodenal segment of the adult, male, unexcited albino rat responds both qualitatively and quantitatively to stimulation by 0.1 M. sodium carbonate by shortening or contraction, and in this paper an attempt is made to localize the intestinal mechanism which is primarily affected. This localization factor is essential for an understanding of the reversal of the reaction to carbonate given by the duodenal segment from a rat excited before killing.

Now the normal response to the carbonate is either due to an action on the musculature or on some part of the nervous system of the segment studied.

Although Meigs (3), (4) claims from his studies on the osmotic properties of smooth muscle that a cellular exosmosis accompanies the contraction of this tissue, it is difficult to understand how excitement can cause a reversal of the normal contraction response to carbonate on the basis of an osmotic effect. Moreover the relatively meager increase in concentration of the 4 cc. of Tyrode's solution in which the segment is suspended, by the addition of but 0.25 cc. of a 0.1 M. solution of sodium carbonate, practically negatives the idea of an osmotic effect as the primary factor in the response.

The other suggestion with regard to the musculature as the mechanism primarily responding to the carbonate is the possibility of a direct stimulation of this tissue: for since it has been demonstrated by Loeb (5), (6) that any increase in the sodium: calcium ratio normally present leads to hyperirritability of nervous and muscular tissues, it might be supposed that by the addition of extra sodium as carbonate this ratio is increased not only by the absolute increase in this element, but also by a partial removal of calcium through precipitation. Indisputable evidence, however, that the calcium-sodium antagonism is *the* impor-

tant factor in intestinal instability is as yet lacking (7). Moreover, inasmuch as on the addition of carbonate the segments from excited animals *relax*, while the segments from unexcited animals *contract*, it is difficult to believe that both pieces of rhythmically contracting, and hence potentially irritable smooth muscle tissue would react in opposite manners to the same amount of the same stimulating substance, whatever its nature, if the reaction was primarily a direct stimulation of the muscle (1). In addition Gaskell's (8) statement that "rhythmic activity is an intrinsic property of involuntary muscle which becomes manifest when that muscle is in a condition of tone; this is dependent upon both suitable chemical conditions and proper nervous control," taken in conjunction with the previous statement, supports the belief that the intestinal musculature is not the mechanism primarily stimulated by sodium carbonate.

Turning now to the nerve elements concerned in the activity of the duodenal segment, certain experimental data have been obtained which tend to support the opinion that this is the intestinal mechanism primarily affected. The problem was attacked by a method of differential physiological analysis using cocain and nicotine as reagents.

Veley and Waller (9) have shown that under certain conditions cocain inhibits contraction of skeletal muscles, and Dixon (10) has stated on slight evidence that the sympathetic nerve endings in blood vessels cannot be paralyzed by this drug. Nevertheless, our tracings here given show that under the conditions we established, cocain does not cause complete inhibition of the muscular activity of the duodenal segment (chart 1, *C*; chart 2, *E*). Moreover, Prus (11) in studies of the heart, and Frölich and Loewi (12), have given definite evidence that this drug does act on nerve endings, while Bayliss and Starling (13) have shown that some part of the nervous conducting system is paralyzed when cocain is applied directly to the intestine; and since rhythmic contractions still occur after cocain, the absence of response to stimuli in their experiments cannot be attributed to a paralysis of the musculature but must be referred to the nervous system.

It would be expected, then that if the response of the duodenal segment to carbonate is due to a stimulation of the nervous mechanism, this response would not be obtained in its normal type after treating the segment with cocain. To test this hypothesis a segment was prepared in the usual way and allowed to trace the record of its response to 0.25 cc. 0.1 M. sodium carbonate as described in the preceding paper (2). One-tenth of 1 cc. of a 1 per cent solution of cocain hydrochloride

was then added to the 4 cc. of Tyrode's solution in the cell in which the segment was suspended, in successive 0.02 cc. portions at intervals of about 1 minute. The first effect was a marked shortening of the segment accompanied by an increased amplitude of the rhythmic contractions. Both of these responses soon passed away and the segment relaxed to its original condition of tone, but continued to trace rhythmical contractions of a low amplitude. The cocain-containing Tyrode's solution was then washed from the cell and segment and replaced by 4 cc. of fresh oxygenated Tyrode's to which 0.02 cc. of the cocain was added. Usually no changes occurred in either tone or amplitude of contraction as a result of this treatment. After a preliminary tracing

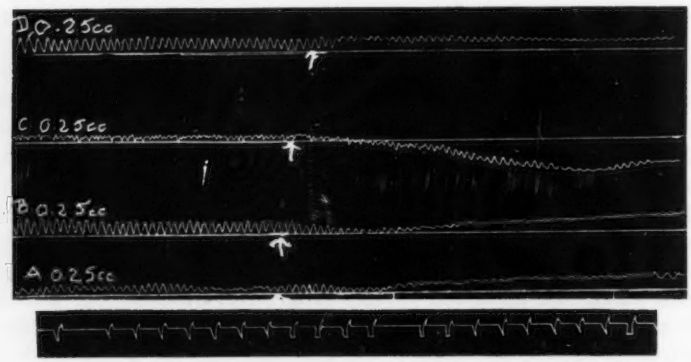

Chart 1. The effect of cocain on the response of the isolated duodenal segment to 0.25 cc. 0.1 M. sodium carbonate. *A–B*, carbonate stimulation before cocain; *C*, carbonate effect after cocain; *D*, carbonate stimulation ten minutes after *C*.

of the movements of the intestine treated in this manner had been recorded just above the records obtained by carbonate stimulation, 0.25 cc. of the 0.1 M. sodium carbonate was added to the solution surrounding the segment. Instead of the characteristic shortening of the segment there occurred a slight relaxation, the trace falling below the original base line from which the response of the untreated segment had been elicited. On chart 1, tracings *A* and *B* show the initial response to carbonate; tracing *C* shows the response after treatment with cocain, and tracing *D*, the recovery to normal type of response 10 minutes after tracing *C* had been made. The tracings amply support the hypothesis, since the muscle shows by its rhythmical contractions

that it is in a condition of tone and potentially able to respond in a normal manner to stimulation, and is only prevented from such response because of the inability of the stimulant to excite the initiating mechanism. The relaxation observed subsequent to the carbonate stimulation will be discussed presently. A point of possible practical clinical application is brought out in the apparent release from the cocain poisoning by the application of the relatively weak solution of sodium carbonate. A clinical study of the effect of sodium carbonate treatment in cases of cocain poisoning would be interesting.

Langley and Dickenson (14) have shown that nicotine in moderate doses prevents the passage of the nerve impulse through the sympathetic ganglia and does not affect the peripheral endings of the fibers in moderate doses, nor prevent the passage of the impulse from ganglion to terminations of the nerves, the effect probably being at the synapse. Bayliss and Starling (13) and others have also shown that after the intravenous injection of nicotine the stimulation of the so-called extrinsic nerves to the intestine fails to produce the characteristic response. Moroever, Bunch (15) has shown that nicotine does not abolish the rhythmic contractions of the intestine and my tracings substantiate this (chart 2, *C* and *D*). Hence we can exclude the action of nicotine on the muscle tissue as used here, and would expect to get the normal type of response to carbonate stimulation after application of the compound to the intestine.

To test this point another segment was taken and instead of being subjected to cocain was similarly treated with 0.1 cc. of a 1 per cent solution of nicotine in successive 0.02 cc. portions at 1 minute intervals. A different result was obtained. After the preliminary shortening of the segment and subsequent relaxation to the original base line the amplitude of the rhythmical contractions remained approximately the same. Even much larger doses of the drug failed to inhibit the ultimate recurrence within a few minutes of the tone and amplitude presented at the beginning of the experiment. When 0.25 cc. of the 0.1 M. sodium carbonate was added to the nicotine-containing solution in the cell the usual response to that substance was obtained. In chart 2, tracings *A* and *B* are the records of the preliminary tests with carbonate, and tracings *C* and *D* are the tracings made after the nicotine poisoning. Tracing *E* is a record made by the same segment after replacing the nicotine-containing Tyrode's with a fresh solution, treating as described with cocain, and stimulated by 0.25 cc. sodium carbonate. The lack of the normal response is evident. In all of the

experiments on the nicotinized segments the carbonate applications were made within 5 minutes of the last addition of nicotine to the solution in which the segment was suspended.

These experiments then support the opinion that the intestinal nervous mechanism is the primary point of stimulation by sodium carbonate.

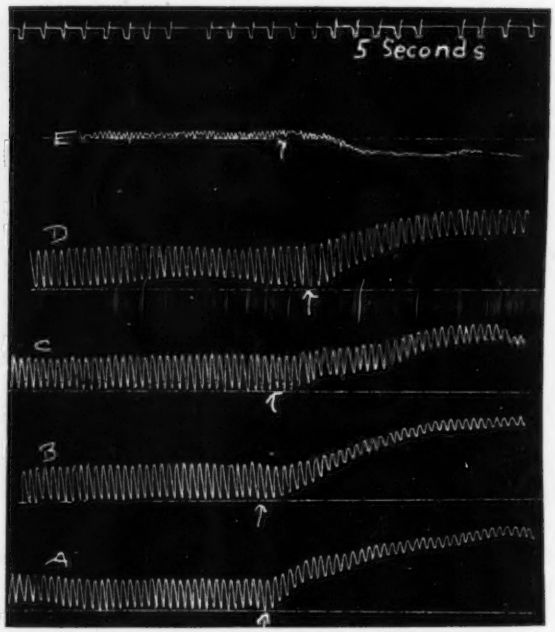

Chart 2. The effect of nicotine on the response of the isolated duodenal segment to 0.25 cc. 0.1 M. sodium carbonate. *A–B*, carbonate stimulation before nicotine; *C–D*, carbonate stimulation after nicotine; *E*, carbonate effect after cocain.

The reversal of reaction to carbonate in excited animals is also explicable on this basis. It has been shown by Bayliss and Starling (13) that stimulation of the superior splanchnic nerve causes an inhibition of intestinal contraction, a relaxation or decrease of tone. Cannon (16) has shown that an effect of excitement is the passage of impulses over the splanchnics to the intestine, and Hatai and I (1) have demon-

strated that both excitement and direct splanchnic stimulation cause a relaxation of the isolated duodenal segment of the albino rat when treated by sodium carbonate. It is generally admitted that the splanchnic nerves convey inhibitory stimuli to the "endodermal" musculature of the small intestine (8). Correlating these facts with Sherrington's (17) conception of the "prepotent" stimulus and with Bayliss and Starling's (13) findings that a motor effect through vagal stimulation is not always obtained unless the intestine be separated from impulses coming in over the splanchnics, we can conclude that the previous excitation of the rat has induced a "prepotent" splanchnic potential condition so that on application of the carbonate a relaxation occurs by virtue of the stimulation of the nerve fibers or endings of the inhibitory splanchnic nerve. In this connection the relaxation subsequent to carbonate application is probably due to the stimulation of the splanchnic endings or fibers which have been released from the cocain before the motor or vagal mechanism, since Dixon (10) has demonstrated that the drug tends to paralyze the motor fibers when directly applied before it acts upon the dilator or inhibitory fibers and hence their release would be delayed over those of the latter system.

It has been shown by Bayliss and Starling (13) that the vagus is the motor nerve to the small intestine, the last cell stations of which are in the gut (18), while the last cell station of the superior splanchnic is in the superior mesenteric ganglion (8). Hence from the fact that after nicotine poisoning we obtain a contraction on carbonate stimulation similar to that obtainable by extrinsic vagal stimulation when the intestine is severed from its splanchnic connectors, and not treated with nicotine, no other conclusion can be drawn but that the carbonate acts either on the intestinal vagal ganglionic cells or the post-ganglionic fibers or endings; and if we accept the classification of the neural regulation of the intestine into intrinsic and extrinsic factors as a matter of convenience rather than as a matter of fact, we can consider that the intestinal mechanism primarily stimulated by sodium carbonate in the normal, unexcited, adult male albino rat is the motor mechanism mediated by the vagus as the extrinsic nerve; while in the excited animal the stimulation is effective through the inhibitory mechanism mediated by the more truly extrinsic nerves, the splanchnics, thus differentiating these activities from the so-called intrinsic mechanism involved in the myenteric reflex.

These and other allied phenomena lead one to the opinion that the neural regulation of the intestine can be conceived to be conditioned by

the nature of the chemical constituents impinging upon the part of that system contained within the intestinal wall. It is but natural that since the endodermic structures are phylogenetically the least removed from the primitive protoplasmic state that they should be the tissues to the greatest extent dependent upon the primitive (chemical) methods of regulation. For although we may agree in part with Bernard (19) that the original structural unit was essentially nervous in function, in that it possessed the properties of irritability and conduction, yet the controlling factors were, and still are, absolutely chemical or in last analysis physical. On the other hand the ectodermic structures and their mesodermic derivatives by virtue of their greater adaptive advance from the primitive state have had their dependence upon the simple chemical regulation transformed into a predominantly neural regulation, which is however in last analysis chemical. It is in fact a change from an immediate chemical stimulation to one of distant chemical stimulation mediated by the nervous system. I cannot look upon this differentiation in the strictly teleological sense as expressed by Hooker (20), who attributes the predominance of the chemical regulation of the glands and deeper tissues generally to a lesser significance of the reaction time; but feel that this differentiation from within outwards, from an immediate chemical regulation to a chemical regulation that is mediated through nerve factors, is largely due to developmental differences in response to differences in the type of environmental phenomena coming in contact with the types of tissue in question.

SUMMARY AND CONCLUSIONS

Evidence is presented leading to the opinion that the intestinal mechanism primarily stimulated by sodium carbonate when applied to the isolated duodenal segment of the albino rat suspended in oxygenated Tyrode's solution at 39°C. is neural and not muscular; and that this stimulation of the segment from the normal, adult, unexcited, male animal is expressed through the mechanism ordinarily mediated by the vagus, while the stimulation of the segment from excited rats is effected through splanchnic endings or fibers. Since these two sets of nerves are conveniently differentiated from the neural mechanism concerned with the myenteric reflex by the term extrinsic it can be said that the carbonate stimulation is primarily transmitted through the mechanisms mediated by the extrinsic nerve supply to the intestine.

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THE INVOLUTION OF THE UTERUS FOLLOWING LABOR AND THE INFLUENCE OF CASTRATION AND SUCKLING ON THE PROCESS OF INVOLUTION

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In these investigations we have studied the retrogression of the uterus following labor in a quantitative way; we compared with the behavior of the uterus as a whole the retrogression curves of its component tissues, and especially did we analyze the effect of suckling and of castration on the retrogression of the uterus. Observations of clinicians have shown that the process of nursing aids the involution and that on the contrary lack of nursing may retard it. Most authors assume that the influence of suckling on the involution of the uterus is due to a reflex mechanism which either through the stimulation of the sensory nerves of the mamilla or through the process of withdrawal of milk causes a contraction of the uterus. This contraction is assumed to favor the retrogression of the uterine muscle.

It has furthermore been established that the involution does not depend upon a destruction of the uterine muscle fibers but upon a diminution in the size of the individual fiber. Inasmuch as our work is of a different character from the aforementioned investigations it will not be necessary to enter here into a detailed discussion of the literature of involution (1).

We used for our studies rat and guinea pig. We examined the uterus about ten hours, and two, seven, fourteen, twenty-one, twenty-eight and thirty-five days after labor. In each case we examined from the second day on at each term four animals in each series, a normal nursing and a normal non-nursing animal, and a castrated nursing and a castrated non-nursing animal. Various parts of the uterus were fixed in Zenker's solution and measurements were made in the stained specimens. Altogether somewhat more than fifty animals in which the time of labor had been observed were used in these investigations.

I: a. Changes in the thickness of the wall of the uterus following labor in the rat. The curves of figure 1 represent the changes in the normal, castrated, nursing and non-nursing rat. In the normal (non-castrated) nursing rat (curve *a*) the decrease in size is most intense in the first two days following labor; the decrease continues during the first week, although with less intensity and then it remains approximately unchanged, although some minor variations do occur. These latter do not appear to have any definite relation to the differences in the weight of the individual rats examined.

In the normal, non-nursing rat the curve (curve *b*) is of a similar character. Here also the decrease is most marked in the first two days and it continues with decreasing intensity until approximately the seventh day. No further important changes take place in the following four weeks. Curve *b* differs from curve *a* in that the former does not reach the low level of curve *a*. As early as two days after labor some difference in the two curves is noticeable; the decrease is slightly less marked in the non-nursing animal. After seven days the difference is still greater. It remains about the same during the next two weeks; in our case reaches a maximum after three weeks; this, however, may be accidental. Following the twenty-eighth day the curves begin to approach each other.

We may therefore conclude that the process of nursing causes an increase in the retrogressive changes in the uterus in the rat, that this increase becomes noticeable in the first few days after labor and is fully developed one week after labor. Curve *c* shows the changes in the castrated, nursing rat. This curve is similar to curves *a* and *b*, but the decrease in size is steeper in the first two days and thus soon a lower level is reached. The decrease continues with lessened intensity during the first week; and in contradistinction to the non-castrated animals it continues till the end of the second week, although at a still less rapid rate. The atrophy caused by castration is therefore added to the retrogressive changes which follow labor; as a result of castration the retrogressive changes are not only more intense, but they continue over a somewhat longer period of time.

In the castrated, non-nursing rat (curve *d*) the changes are in the first week approximately the same as in the castrated nursing animal. From the end of the second to the end of the fifth week the curve ascends slightly; the loss in size is here slightly less marked than in the nursing, castrated rat (curve *c*). In the castrated rat the effect of nursing in reducing the size of the uterus becomes therefore noticeable at a later

date and is considerably less marked than in the normal animal. The effect of castration in diminishing the size of the uterus is more marked than the effect of nursing; the uterus is smaller in the castrated non-nursing than in the non-castrated nursing rat. In the castrated, nursing rat the uterus is smallest; here a summation of the effect of castration and nursing has taken place. In the later periods of observation the uterus in the castrated rats is not only very thin, but it also assumes a yellowish color; it seems as if the atrophy led to pigment changes comparable to those found in the heart and liver in certain atrophic conditions.

We find thus that castration brings about a condition unfavorable for the full development of the effects which are caused by the lack of nursing in the normal rat.

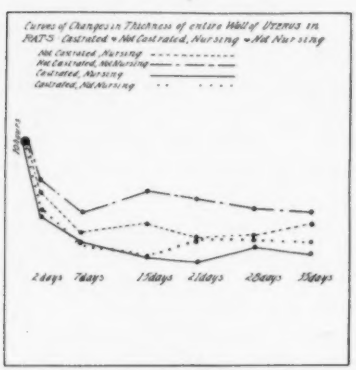

Fig. 1

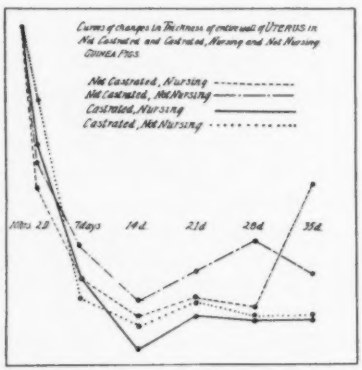

Fig. 2

-------- *a* — - - - *b* ——— *c* ..... *d*

I: b. Changes in the thickness of the wall of the uterus following labor in the guinea pig. In the normal non-nursing guinea pig the curve representing the change in the thickness of the entire wall (curve *a*, fig. 2) of the uterus is on the whole similar to the corresponding curve in the rat. However, in the guinea pig the decrease in the first week is absolutely and relatively greater than in the rat. Furthermore the retrogression continues in the guinea pig over a longer period, reaching a maximum on the fourteenth day. The curve of the normal, non-nursing guinea pig (curve *b*) resembles in both respects that of the nursing animal; but as in the rat, the uterus is larger in the non-nursing animal.

The difference is noticeable in the next few days following labor and reaches a maximum between the third and fourth week after labor. Absolutely the difference in the size of the nursing and the non-nursing guinea pig is similar to that of the rat, but relatively the effect of the lack of nursing is somewhat greater in the rat. In both species the difference between the nursing and the non-nursing animals either becomes very small or disappears five weeks after labor.

In the castrated guinea pigs retrogression takes a similar course to that in the normal animals during the first seven days. In the second week, however, the retrogression becomes more intense in the castrated animal. The curve (*c*) reaches here a lower level fourteen days after labor and from now on remains constantly at this lower level, just as it did in the rat. The curve of the non-nursing castrated guinea pig (curve *d*) is similar to that of the nursing castrated guinea pig, but again remains at a somewhat higher level. Again the uterus shows less retrogression in the non-nursing animal. However, as in the case of the castrated rat so also in the castrated guinea pig the difference between the size of the nursing and the non-nursing animals is much smaller than in the corresponding non-castrated animals.

We may therefore draw the conclusion that castration diminishes the effect of the lack of nursing upon the retrogression of the uterus. In both the castrated guinea pig and rat series, a relatively slight difference is still present between the nursing and the non-nursing animals; in both cases this difference appears somewhat later in the castrated animals. Indeed the difference is so small in the castrated series that we may consider whether it is not altogether accidental, especially in view of the fact that the animals in the castrated non-nursing series happened to be somewhat heavier. This applies to the rat series more than to the guinea pig series. We believe it however more probable that the difference, while considerably less marked, is nevertheless real in the castrated series, because the differences in the size of the animals do not run parallel to the curves *c* and *d* in the rat and guinea pig; especially the curve in the guinea pig seems to be independent of these variations in weight.

The fact that the difference between the nursing and the non-nursing series diminishes in the castrated series might suggest that the lack of nursing affects primarily the conditions of the ovaries and acts only secondarily through the ovaries upon the uterus. This is, however, not a probable conclusion. It is much more probable that the influence exerted by the ovaries upon the uterus makes the latter more receptive

for the effect of the nursing or the lack of it. This latter interpretation is necessary if we interpret the difference in the curves of the nursing and the non-nursing animals in the castrated series as real. If we compare in the guinea pig the curves of the castrated (curves *c* and *d*, fig. 2) and non-castrated (curves *a* and *b*, fig. 2) animals we find a more even course in the castrated series; this is probaby due to the fact that in the castrated guinea pig those variations which are due to ovulation are eliminated.

In the rat, on the other hand, such variations do not occur in the nursing animals; and in the non-nursing animals they occur at such short intervals that the expression of the full strength of these variations is interfered with.

We shall now consider the changes in the various constituents of the uterine wall in order to determine whether these changes are limited to certain structures or affect all of them in an equally marked way.

II: a. The surface epithelium following labor in the rat. The changes in the surface epithelium are represented in the curves of figure 3. These curves give only an incomplete picture of the actual changes which take place, inasmuch as they consider only the variations in the height and not in width of the epithelial cells. The same holds good of the corresponding curves in the guinea pig (fig. 4).

Soon after labor the surface epithelium is high in the rat. After two days a decrease has taken place in the non-nursing as well as in the nursing animals of the normal series. In the nursing individuals the height either remains on this level or falls below it at certain times, while in the non-nursing animals the height increases again in the period following the first two weeks. If we consider in addition the second diameter we notice that from the seventh day on it is large in the non-nursing and small in the nursing individuals. The difference in the size of the epithelial cells in the two series is therefore greater than is shown in the curves *a* and *b*. These differences run on the whole parallel to the variations which we found in the size of the uterus as a whole.

This difference between the surface epithelium of the two series is also apparent, if we compare the number of mitoses. Directly after labor the number of mitoses is very small, it increases considerably in the next two days in both the nursing and the non-nursing animals; from this period on a difference appears in the two series. In the nursing animals mitoses are altogether absent during the next four weeks and they are very rare at five weeks; while in the non-nursing individuals about 6 to 7 mitoses can be seen in each section seven and fifteen

days after labor; and while at three and four weeks only a small number of mitoses can be seen, it rises again considerably after five weeks. We see therefore that the greater size of the epithelium in the non-nursing animals is accompanied by a greater number of mitoses.

In the castrated rats (fig. 3, curves *c* and *d*) the curves fall below those of the normal animals in the course of the first week. This difference between the castrated and the normal animals reaches its full strength at the end of the first week, and with some variations the curves of the castrated rats remain on the whole below those of the normal ones during the five weeks following labor. Again the height of the epithelium

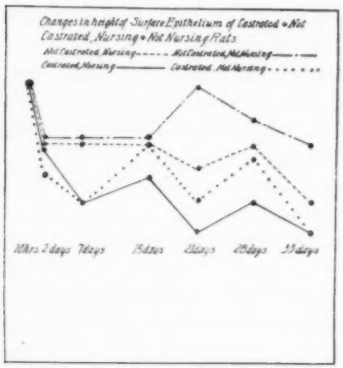
Changes in height of Surface Epithelium of Castrated + Not Castrated, Nursing + Not Nursing Rats.
Not Castrated, Nursing
Not Castrated, Not Nursing
Castrated, Nursing
Castrated, Not Nursing
10hrs 2 days 7days 15days 21days 28days 35days

Fig. 3

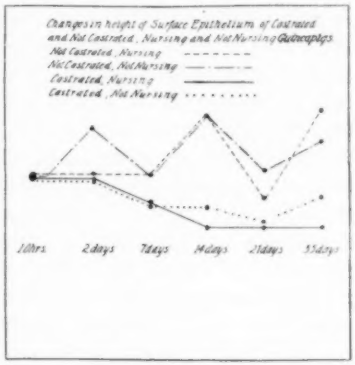
Changes in height of Surface Epithelium of Castrated and Not Castrated, Nursing and Not Nursing Guinea pigs.
Not Castrated, Nursing
Not Castrated, Not Nursing
Castrated, Nursing
Castrated, Not Nursing
10hrs 2days 7days 14days 21days 35days

Fig. 4

-------- *a* —·—·— *b* ——— *c* ..... *d*

is greater in the non-nursing than in the nursing animals, but in the castrated rats the difference between these two series is not so great as in the normal rat.

If in addition we consider the second diameter the difference between the non-nursing and the nursing series in the castrated animals becomes still greater, and the curve of the nursing animals would fall below that of the non-nursing ones also seven and thirty-five days after labor. Likewise the difference between the castrated and the normal rats would be still greater than is shown in the curves.

In the castrated nursing animals the number of mitoses is very small or generally they are missing altogether, while in the castrated non-nursing animals the number of mitoses is usually higher, without however reaching the number of mitoses in the normal, non-nursing series.

We may conclude that in the rat nursing and castration both tend to diminish the size of the surface epithelium, that both effects are additive and that the effect of the nursing or the lack of it does not at all or not exclusively operate through the ovaries, but that the nursing acts directly on the uterus. Again castration diminishes absolutely and relatively the effect of the non-nursing on the uterine epithelium.

II: b. Changes in the surface eithelium following labor in the guinea pig. In the normal nursing (curve *a*, fig. 4) and non-nursing (curve *b*, fig. 4) guinea pig the curves representing the height of the surface epithelium take an approximately parallel course. The greater size of one diameter in the non-nursing animal at seven days after labor may be accidental. Variations in the character of the epithelium in dependence upon periodicity of the sexual cycle are evidently of much greater importance than variations due to nursing or the lack of it. The difference in the size and mitotic activity of the epithelium which we do find later than fourteen days after labor correspond to differences in the sexual periodicity in the individuals of the two series.[1]

Thus in the animals examined three weeks after labor ovulation had taken place in the nursing guinea pig about two and one-half days and in the non-nursing animal about three to four days previously. Accordingly we find in both the surface epithelium rather high and the number of mitoses much greater in the nursing than in the non-nursing individual. In the animals examined four weeks after labor ovulation had occurred approximately ten to thirteen days previously in the nursing and five days previously in the non-nursing animal. Accordingly the curve shows a descent in both, but a more marked one in the nursing animal. In both the number of mitoses is very small in accordance with the stage of the sexual cycle.

In the guinea pigs examined five weeks after labor ovulation had occurred two to three days previously in the nursing and five to six days previously in the non-nursing animal; accordingly the epithelium is somewhat higher in the former than in the latter, and the number of mitoses is considerably greater in the former.

If we turn to the castrated series (curves *c* and *d*) we find a marked decrease in the height of the epithelium in the nursing as well as in the non-nursing animals; in both the decrease begins to become evident in the course of the second week. After three, four and five weeks it is

[1] The structural cyclic changes in the uterine mucosa have been described with some detail by one of us in a previous paper (Leo Loeb, Biol. Bull., 1914, xxvii, 1).

more marked in the nursing than in the non-nursing series; in the latter the size of the epithelium is again somewhat higher. However, even in the castrated guinea pigs the difference is not very great between the nursing and the non-nursing animals. The number of mitoses is rather variable in the castrated guinea pigs; it may become considerable. There does not however seem to be in the castrated series a definite influence of nursing on the number of the mitoses.

While therefore the influence of castration on the size of the surface epithelium is marked in the guinea pig and about of the same order as in the rat, the influence of nursing and non-nursing is noticeable only in the rat as far as the normal series is concerned; while in the castrated series a slight influence of the nursing or lack of nursing exists also in the guinea pig. This difference in the behavior of rat and guinea pig depends on the greater influence of the changes due to the sexual cycle in the guinea pig as compared with the rat.

III: a. The uterine glands following labor in the rat. Curve *a* (fig. 5) represents the changes in the length of the uterine gland in the normal, nursing rat. Directly after labor the glands are short, probably in consequence of the stretching in the pregnant uterus and of the loss of part of the uterine mucosa during labor. There is a rapid increase in size which reaches a maximum seven days after labor. In the first two days the increase is more rapid than later. Five weeks after labor the length is approximately the same as after seven days. But in this period a secondary decrease takes place which reaches a maximum three weeks after labor. It is doubtful what significance should be attached to this secondary minor variation.

In the non-nursing animal (curve *b*, fig. 5) the curve shows a similar course to curve *a* in the first seven days, but it reaches a slightly higher level as early as two days after labor. From the end of the first to the end of the fifth week the increase in the length of the gland is very much greater in the non-nursing rats. We see thus a marked effect of nursing or of the lack of it. This difference between the two curves becomes still more definite, if we not only consider the length of the glands, but also their diameter and the size of the gland cells, both being greater in the case of the non-nursing rats. The difference is also very marked as far as the mitotic activity is concerned. From the seventh to the thirty-fifth day mitoses in the glands are usually missing in the nursing normal rats; they are present in variable numbers in the non-nursing animals. At two days after labor, however, mitoses are frequent in all the four animals; this is probably due to the regenerative processes which take place soon after labor.

In the castrated series the increase in size is just as marked in the first two days, but no further increase takes place in these animals. On the contrary the curve descends again, showing a course parallel to that of the normal, nursing rats, always remaining below that of the latter. Similar is the curve of the castrated, non-nursing rats; it is higher than that of castrated, nursing animals, but much lower than that of the normal non-nursing rats.

There is thus still noticeable an influence of the nursing or rather of the lack of nursing in the castrated animals, but again it is less marked than in the normal rats. Correspondingly we find, following the first week, usually a greater diameter of the glands and a greater size of the

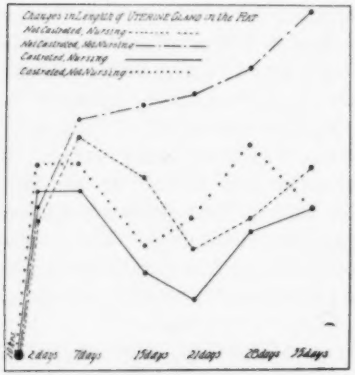

Fig. 5.

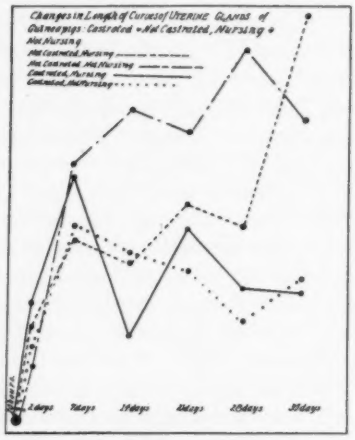

Fig. 6.

..... *a* — — — — *b* ——— *c* ..... *d*

gland cells in the non-nursing animals even in the castrated series. Likewise we usually but not invariably find a larger number of mitoses in the non-nursing animals of the castrated series as compared with the nursing animals of the same series. Two days after labor there are many mitoses present in the glands in the castrated nursing as well as non-nursing animals; in all probability they are of a regenerative character. The size of the gland cells and the number of mitoses in these cells in the non-nursing normal animals are usually greater than in non-nursing castrated animals. As far, however, as the number of mitoses is concerned, this does not hold good in every case.

III: b. Changes in the uterine glands following labor in the guinea pig. In the guinea pig the curves in the normal series (fig. 6, *a* and *b*) are similar to those in the rat, except that in the guinea pig five weeks after labor the typical difference between the nursing and non-nursing animal no longer exists. Again the uterine glands are very small soon after labor and grow rapidly in the first week. During this period the non-nursing overtake the nursing animals and the difference remains very marked until the latter part of the fifth week is approached.

Three, four and five weeks after labor the difference in the size of the gland cells and in the number of mitoses in the various parts of the glands is in accordance with the difference in the stage of the sexual cycle in the respective animals. The normal sexual cycle would thus tend to increase somewhat the length of the uterine glands in the non-nursing animals three, four and five weeks after labor. These effects however are merely added to the changes caused by the effect of nursing which are already pronounced as early as seven and fourteen days after labor.

In the castrated series the curves (fig. 6, *c* and *d*) are at first similar to those in the normal series except that in the first week the glands are here longer in the nursing than in the non-nursing animals. From then on the curves decline below those in the normal series, the difference between the two series becoming more intense with advancing time. In the castrated series the typical effect of non-nursing is absent. From the third week on the size of the gland cells and the number of mitoses in this series is smaller than in the normal series.

IV: a and b. The uterine mucosa in the rat and guinea pig following labor. The changes in the thickness of the uterine mucosa run parallel to those in the uterine gland as is evident, if we compare figures 5 and 7 (rat) with figures 6 and 8 (guinea pig). The glands are embedded in the mucosa and both these structures vary concomitantly. A detailed discussion of these curves is therefore unnecessary. The only noticeable difference between the changes in glands and uterine mucosa is found in the rat in the first few days after labor. While the glands increase here in size in the first two days, the thickness of the mucosa decreases in the same period of time; from then on the curves take an approximately parallel course.

If we study the character of the mucosa, we find in the rat the following conditions: Directly after labor there has been left only a narrow strip of fibrous mucosa in which the connective tissue cells are small. As a result of the preceding labor the tissue has become diffusely ede-

matous; the number of lymphocytes and polynuclear leucocytes present is very small. We observe a limited number of mitoses. Two days after labor the mucosa is still fibrous throughout and the connective tissue cells are small; edema is absent. In the normal animals the number of mitoses is greater than in the castrated animals. From now on certain changes take place gradually.

In the normal nursing animals the mucosa becomes transformed into a cellular tissue rich in large fibroblasts, while in the other animals the mucosa remains fibrous.

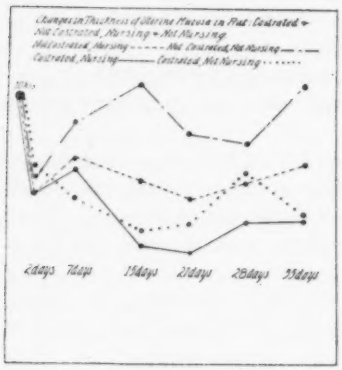

Fig. 7

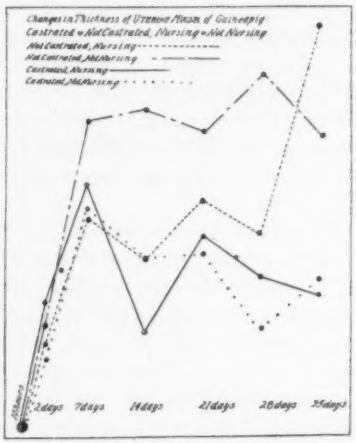

Fig. 8

-------- *a* — - - - — *b* ——— *c* ..... *d*

In the castrated rats the cells become smaller than in the normal ones, first in the nursing and at last also in the non-nursing rats. The mucosa is usually more fibrous in the castrated animals. In the non-nursing animals the number of lymphocytes, and especially of polynuclear leucocytes, is greater than in the nursing ones and this is perhaps somewhat more marked in the normal than in the castrated series.

The number of mitoses in connective tissue cells is variable, but on the whole it is greater in the normal than in the castrated rats, and toward the latter period of the experiment it is greater in the non-nursing than in the nursing animals. In the guinea pig also the mucosa is narrow and fibrous soon after labor. In both rat and guinea pig regenerative processes take place in the next few days following labor, the

connective tissue cells everywhere increase in size and mitoses are found in the connective tissue of the mucosa in all animals two days after labor; but they are more numerous in the normal than in the castrated series. The mitoses found at that time are in the rat as well as in the guinea pig of a regenerative character. In the castrated series the mucosa remains fibrous throughout. The connective tissue cells become gradually smaller, and undergo this change more rapidly in the nursing than in the non-nursing animals in a similar way to the rat; only isolated mitoses are found. In the normal series the condition of the mucosa is influenced by the cyclic changes which are initiated in the ovary and to a great extent depend upon the function of the corpus luteum, as one of us has shown previously. Thus we find after seven days predecidual tissue in the mucosa of both normal animals, the number of mitoses corresponding to the state of the cycle. These proliferative changes are due to the influence of the corpora lutea which were formed spontaneously soon after labor in nursing and in non-nursing animals. Two weeks after labor both animals are again in a stage of the sexual cycle at which proliferative activities have ceased; the mucosa again has become fibrous. After three weeks we find more activity in the non-nursing than in the nursing animal, because in the former ovulation has taken place about three to four days previously, while in the nursing animal ovulation had taken place only two and one-half days previously. But even in the nursing animals we find here the beginning of a new proliferative activity. After four weeks the condition of the mucosa in the nursing animal indicates that it had just passed through an active period of proliferation; while mitotic proliferation had ceased, the mucosa is still cellular.

In the non-nursing animal mitoses are present in accordance with the fact that in this guinea pig ovulation had taken place about five days previously. After five weeks we find in the non-nursing animal predecidual tissue formation in accordance with the stage of the sexual cycle, an ovulation having taken place five to six days previously, while in the nursing animal the ovulation had taken place more recently and the changes are consequently in a very early stage.

Edema is found more often in the mucosa of the normal than of the castrated animals as should be expected considering the vascular changes which are connected with the sexual cycle. Infiltration with lymphocytes and polynuclear leucocytes is not of much importance in the guinea pigs; it accompanies usually an edematous condition of the mucosa.

We find then that, in contrast to what we find in the rat, the cyclic changes play an important part in determining the condition of the mucosa in the guinea pig, and furthermore we find that in the guinea pig lactation does not only not prevent cyclic changes in the ovary of the non-castrated animals, but that it even permits the cyclic changes in the uterus to proceed. This is quite evident after seven days; but there are also indications that at later stages ovulation was followed by cyclic proliferative changes in the mucosa, although at this time the changes are not marked, because the nursing animals happened to be found at a stage of the sexual cycle when proliferation had already either ceased or was just beginning to commence. However, the changes in the mucosa, but especially the changes in the epithelium of surface and glands which we have described above, make it certain that even in the nursing guinea pig the sexual cycle proceeds in the uterus.

At this place we shall refer to some changes which are occasionally found in various parts of the connective tissue. In the rat we may observe some hemorrhage especially in the connective tissue of the mesentery in the first week following labor. In the following week we notice cells containing remnants of blood pigment in various parts of the connective tissue, namely, in the mucosa, muscle and mesentery.

In addition, we find in the first seven days following labor some giant cells in the mesentery. They have either migrated here from the placenta or they originated in the mesentery in connection with the formation of the placenta.

In non-castrated guinea pigs we observed in two cases (two and three weeks following labor) remnants of decidua in the mucosa directly under the surface epithelium. Evidently in these cases an incomplete separation of the placenta took place; subsequently the epithelium regenerated over the left-back decidual tissue and the latter became thus incorporated in the mucosa.

V: a. The uterine muscle following labor in the rat. Instead of treating separately the longitudinal and circular muscle layer, we consider the sum of both in the rat as well as in the guinea pig (figs. 9 and 10). During pregnancy a hypertrophy of the muscle layers takes place. Soon after labor a rapid decrease sets in (curve *a*, fig. 9), which gradually becomes slower. A minimum is reached at the end of the first week and from then on the size remains approximately at the same level with some minor variations. The initial decrease in the size of the uterine muscle in the rat is steeper than that of the other uterine tissues. It is approximately equally steep in the guinea pig. The

curves are therefore in rat and guinea pig more similar than those of other tissues. However the minimum is again reached in the rat at an earlier date (7 days) than in the guinea pig (2 weeks). The curve in the non-nursing animals (curve *b*) follows a similar course, but as in the case of other tissues the size of the muscle tissue is greater in the non-nursing animals. This difference between the nursing and the non-nursing rats becomes marked only after the end of the first week; it decreases again in the fifth week after labor. The difference between the two curves is of a similar order to that found in the case of the uterine mucosa.

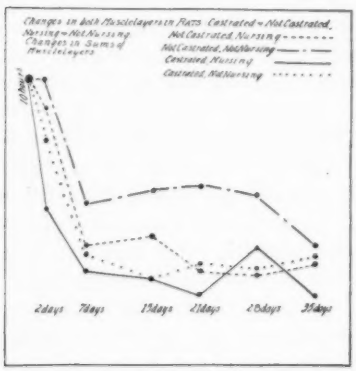

Fig. 9

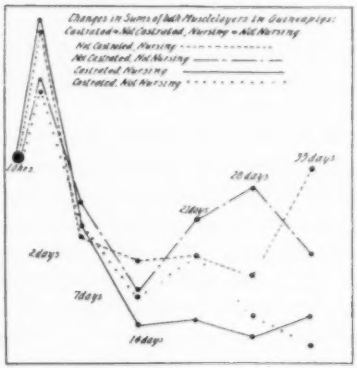

Fig. 10

-------- *a* —·—·— *b* ——— *c* ..... *d*

In the castrated series (curves *c* and *d*) the decline in size is still steeper than in the normal series. Seven days after labor a minimum is reached which is somewhat lower in the castrated than in the normal rats. With certain slight variations this lower level is maintained. In the castrated series no distinct difference is noticeable between the nursing and the non-nursing animals.

Mitotic proliferation in the muscle layer. Directly after labor no mitoses are found in the unstriated muscle cells, but in the next few days a very marked mitotic proliferation sets in in all the animals, even in the castrated rats. At the end of the first week it has almost come to an end. This proliferation is probably the response of the tissue to the changes which occurred in the uterus during labor; the mitoses are therefore probably of a regenerative character. From the end of the

first week on mitoses become very rare, but those that do occur are not found in the castrated, but merely in the normal and especially, it seems, in the non-nursing animal. Castration therefore in causing atrophy of the muscle adds a factor unfavorable for mitotic proliferation.

Castration not only diminishes the mitotic proliferation in the unstriated muscle cells, but it also greatly diminishes the size of the individual cells. This holds good for the whole period of observation from two days on to five weeks following labor. In general the reduction of the uterine muscle following labor is due to a decrease in the size of the individual cells.

In a similar way the muscle cells of the guinea pig decrease in size in the days directly following labor. Castration leads also in the guinea pig to an additional decrease in the size of the muscle cells. This becomes especially marked in the time following the first two weeks after castration.

V: b. Changes in the uterine muscle layers in the guinea pig following labor. (Fig. 10.) In the guinea pig (curve *a*) the muscle layers gain somewhat in thickness in the first two days following labor. This is presumably due to the contraction of the uterine wall soon after labor and the gain is therefore probably more apparent than real; but after this preliminary change a sudden decrease sets in which reaches a maximum at fourteen days; from then on the curve remains even with only slight variations. There occurs again a sudden rise after five weeks, which may in part be of an accidental character. In the non-nursing guinea pigs (curve *b*) the muscle layer is thicker than in the nursing animals after three and four weeks; but two weeks after labor we notice a perhaps accidental fall of curve *b* below that of the nursing animal.

In the castrated guinea pigs the curves *c* and *d* follow a similar course, but as early as two weeks after labor they have reached a lower level in this series and this difference is maintained in the following weeks.

After the first week the curve *d* of the non-nursing animal begins to be on a higher level than the curve *c* of the nursing guinea pig. In both the guinea pig and the rat the thickness of the uterine muscle layer in the castrated non-nursing animals is generally slightly below, but sometimes equal to that in the nursing non-castrated animals. This is in contrast to the uterine mucosa and surface epithelium where the size of the tissues in the castrated, non-nursing animals is usually more distinctly below that of the tissues in the non-castrated, nursing animals.

The mitotic proliferation in the muscle layer follows a similar course in the guinea pig and in the rat. Directly after labor mitoses are absent.

Soon there sets in quite a noticeable mitotic activity in all the individuals, nursing and non-nursing, castrated and non-castrated; this proliferation is probably of a regenerative character. But in the guinea pig the proliferation continues, although with diminished intensity and not equally strong in all individuals during the first three weeks. After seven days the mitoses happen to be more frequent in the castrated animals, but from then on the effect of castration in reducing the mitotic activity becomes evident. After two and three weeks mitoses are much more frequent in the non-castrated guinea pigs. They are usually absent, or only exceptionally present, in the castrated animals. After four weeks they are absent also in the normal animals, but after five weeks they are again more frequent in the non-castrated guinea pigs. Whether or not mitotic proliferation in the muscle layer shows a cyclic variation, being more frequent in the first three days after ovulation, has not yet been determined by us.

In the rat we found some giant cells in the connective tissue of the mesentery in the period following labor; in a similar way we find in the guinea pig giant cells in the connective tissue separating the two muscle layers. These giant cells can be observed during the first two weeks following labor; subsequently they disappear. In the guinea pig they apparently have the power of destroying the endothelium of some blood vessels and thus perhaps of causing slight hemorrhages. Occasionally blood pigment cells are found in the same location, namely, in the connective tissue between the two muscle layers.

VI: Changes in the connective tissue in the outer wall of the uterus following labor in the rat and guinea pig. If we deduct from the figures for the combined diameter of the two muscle layers and the adjoining connective tissue the sum of the two muscle layers, we obtain the diameter of the connective tissue in the outer wall of the uterus. In constructing the corresponding curves we find in the case of the guinea pig an almost perfect identity of all four curves, while in the rat they are very similar in the castrated and the non-castrated, nursing and non-nursing animals; a difference which is probably accidental is here found merely on the fifteenth day.

We may conclude that the outer connective tissue does not play a significant part in the variations which are caused by the presence or absence of ovarian function and by the process of nursing.

DISCUSSION

1. In the rat and the guinea pig the effect of nursing the young and of castration on the size ahd structure of the uterus is of a similar order; especially castration diminishes the size of the uterus in a quantitatively similar manner in rat and guinea pig; the effect of nursing or of the lack of it is perhaps slightly greater in the rat than in the guinea pig, although the difference is not very great.

Castration and lack of nursing exert an opposite influence on the uterus. If both interfere with each other, the effect of castration is dominant. Lack of nursing does not prevent the effect of castration, although it seems to modify it slightly. Castration exerts its influence in nursing as well as in non-nursing animals. In castrated animals on the other hand the effect of lack of nursing is much smaller than in normal animals, and in the case of certain tissues it may be abolished altogether. The size of the uterus in non-nursing castrated animals is slightly smaller than in normal nursing animals. There occurs in a limited way a summation of the effects of nursing and of castration.

The effect of nursing on the uterus is not a mediate one acting through the ovaries; on the contrary our results indicate that there is still a slight effect of nursing or lack of nursing present in castrated animals. It is therefore more probable that the changes brought about in the uterus by castration render difficult the realization of the effects of the lack of nursing.

Involution of the uterus takes a somewhat similar course in rat and guinea pig, the decrease is very rapid at first and gradually becomes slower. However, the decrease following labor is much greater in the guinea pig and the minimum in size is reached somewhat later in this animal. The effect of lack of nursing makes itself felt very early, within the first week after labor, and it becomes more marked during the following weeks. The effect of castration—additive to that of involution—appears in the rat in the first week and in the guinea pig in the second week or toward the end of the first week. The effect of castration is reached at the same time as that of involution or of nursing or slightly later.

Involution occurs also in the non-nursing animals and the maximum of involution is reached at about the same time in the nursing and the non-nursing animals; it is merely the intensity of involution in which the nursing and the non-nursing animals differ. Gradually the effect of nursing or the lack of it seems again to disappear. This is indicated by the character of the curves five weeks after labor.

If instead of the uterus as a whole we analyze the effect of nursing and castration on the involution of the component tissues, we find that the various structures of the uterus are essentially affected in a similar manner, but that there are some modifying factors in the case of some tissues. The effect of castration on the size and also on the mitotic proliferation of the surface epithelium is marked in guinea pig and rat. The maximum effects are reached in both species as late as three weeks after labor. The effect of lack of nursing is noticeable in both series in the rat as far as the size of the epithelium, and less clearly as far as the number of mitoses is concerned; but again the effect is diminished by castration. In the guinea pig there is a weak effect of nursing noticeable in the castrated animals; but in the normal series the typical cyclic changes, which, in contradistinction to the rat, are not suspended in the nursing guinea pigs are of much greater significance than the effects due to the nursing, as far as the character of the surface epithelium is concerned. In the first few days after labor changes of a regenerative character influence the proliferation and size of the surface epithelium as well as of muscle cells and connective tissue of the mucosa.

In the case of the uterine glands and mucosa we have not to deal with a real involution inasmuch as (with the exception of the uterine mucosa in the rat) the size of these structures is directly after labor smaller than two days later; but otherwise we find in the case of these tissues the typical effect of nursing and of castration and of the combination of both. Castration diminishes the size of these structures and so does nursing in the non-castrated animals. In the castrated animals the effect of lack of nursing is noticeable only in the rat and not in the guinea pig; but even in the rat it is less marked in the castrated than in the non-castrated animals.

In both guinea pig and rat castration not only leads to a diminution in the size of the gland cells but also to a diminution in the number of mitoses. In addition nursing leads to a diminution of mitoses in the glands in the rat, while in the normal guinea pig the cyclic changes determine to a great extent the number and seat of mitoses in the gland cells at various stages of the cycle. In non-castrated animals the difference between nursing and non-nursing animals is most pronounced in the size of the glands and of the mucosa; both of these structures behave in a very similar manner.

The changes in the mucosa present some interesting features. As far as size is concerned, the effect of nursing and of castration and of a combination of both is typical in rats and with certain restrictions also

in the guinea pig. In castrated guinea pigs, however, the difference between nursing and non-nursing animals disappears as far as the diameter of the mucosa is concerned. In non-castrated nursing rats the mucosa becomes very rich in large fibroblasts. Castration on the other hand tends to increase the fibrous character of the mucosa and to diminish the number of mitoses. Of interest is the observation that in the non-nursing rats the number of lymphocytes and polynuclear leucocytes in the mucosa is greater than in the nursing animals.

In the castrated guinea pigs the mucosa remains fibrous and the connective tissue cells become smaller especially in the nursing animals. In the non-castrated guinea pigs the character of the connective tissue in the mucosa depends mainly on the stage of the sexual cycle of the animals in the nursing as well as in the non-nursing series.

The conditions which we found in the surface epithelium, glands and mucosa in the nursing and the non-nursing, non-castrated guinea pigs, permit the conclusion that cyclic changes not only occur in the ovaries but also in the uterus of these animals. This is in contrast to what we find during pregnancy. Here, as one of us has shown previously (2) the cyclic changes are suspended in the ovaries as well as in the uterus. Through extirpation of the corpora lutea it becomes however possible to cause ovulation and cyclic changes in the ovaries even during pregnancy; but a second mechanism exists in pregnant animals which prevents the corpora lutea from exerting their effect on the uterine mucosa. During pregnancy the latter remains unresponsive to the sensitizing effect of the corpora lutea. In this respect lactation does not have the same effect as pregnancy, as our present experiments prove.

Involution of muscle cells in rats and guinea pigs takes a similar course to the other tissues; the effect of castration, nursing or lack of nursing and castration and of the combination of both is typical in both rats and guinea pigs. Castration as well as involution causes a marked diminution in the size of muscle cells. Directly after labor mitoses of a regenerative character occur in the muscle cells; later mitoses are much more frequent in the non-castrated than in the castrated animals.

We see then that castration affects the size as well as the proliferative activity of all the tissues that compose the uterus. Nursing also affects all the structures, but the influence of castration is more potent than that of nursing or the lack of it.

If we compare the various curves we see furthermore that involution in the normal nursing animal is either intensified (by castration) or

weakened (by lack of nursing), but that both these influences merely lower or elevate the maximum of the curves without altering the time relations. In each kind of animal these three effects take a similar course as far as time relations are concerned. On the other hand the time relations are slightly different in rat and guinea pig.

This suggests the interpretation that possibly both castration and lack of nursing act on the uterus through the same mechanism although in an opposite direction and that the effect of castration dominates, if both influences interfere with each other.

In our experiments we used two kinds of animals, rat and guinea pig, the former representing a type in which ovulation and uterine cyclic changes cease in the nursing animal, while the guinea pig represents a type in which both continue to take place. We saw that on the whole the course of involution, the effect of nursing and non-nursing, the effect of castration and of a combination of these conditions is essentially the same in both types of animals and that the cyclic changes modify merely the retrogressive processes in the uterus in the case of certain tissues.

It is of interest to correlate the difference in the effect of nursing on the cyclic changes in the ovaries and uterus in the guinea pig and the rat with the fact that the former animal nurses a relatively much smaller number of young than the latter, and furthermore that in the guinea pig the young after birth are much more mature and less dependent on the milk of the mother than in the rat. The effect of lactation on the cyclic changes is correspondingly more far reaching in the rat than in the guinea pig, although the retrogressive changes are of a similar order in both.

In the human species, an intermediate condition between that found in guinea pig and rat seems to exist as far as we can judge from the literature; here the occurrence of menstruation (and ovulation?) seems to be variable in different individuals.

SUMMARY

1. Nursing accelerates the involution of the uterus; lack of nursing weakens involution in both rat and guinea pig. Castration intensifies involution.

Involution of the uterus following labor takes a similar course in rat and guinea pig; the changes take place with decreasing intensity and affect all the uterine tissues.

2. The effects of castration and of nursing are added to those of involution. These conditions merely change the intensity of the involution without altering markedly the time relations; the curves representing the involution under these various conditions take therefore a parallel course.

3. Both castration as well as nursing affect all the principal constituents of the uterus and they affect each tissue in an analogous manner; they influence the size of tissues, their proliferative activity and their structure.

4. Castration and lack of nursing exert opposite effects. If both are combined, the effects of castration dominate. In castrated animals the effects of "non-nursing" are much diminished and in the case of certain tissues suspended altogether.

5. In contradistinction to pregnancy which in the guinea pig not only suspends the ovarian cycle, but in addition and independently of the ovarian cycle inhibits the uterine cycle, lactation neither suspends the ovarian nor the uterine cycle. In the nursing rat on the other hand both are suspended. While the cyclic changes which occur in the uterus of the nursing guinea pig modify to some extent the effect of nursing on certain uterine tissues, the course of involution is approximately the same in these species, although they represent different types, as far as the effect of lactation on the periodicity of the sexual cycle is concerned.

6. The effects of nursing or the lack of it are in all probability exerted upon the uterine tissues directly and not indirectly by way of the ovaries. Castration seems to alter the uterine tissues in such a way that they become less responsive to the effects caused by the lack of nursing.

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THE INFLUENCE OF LACTATION ON THE SEXUAL CYCLE IN THE RAT AND GUINEA PIG

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In the course of a study of the retrogression of uterus and mammary gland following labor in guinea pig and rat, it was found necessary to compare the effect of lactation directly on the cyclic changes in the ovary and indirectly on the sexual cycle in other organs also.[1] We shall first discuss our findings in the rat and then in the guinea pig.

The influence of lactation on ovulation in the rat. We compared the ovaries of rats which were allowed to nurse their young and of others from whom the young had been taken away soon after labor. Such pairs of rats were examined at various times during a period extending between less than a day and five weeks following labor.

If we compare the ovaries of the non-nursing and the nursing rats, we find that the former are usually larger; they generally contain a greater number of corpora lutea, and among them we find corpora lutea which are quite young; in a number of cases we found in the non-nursing rats corpora lutea which were in the earliest stage of development. In addition we usually find various kinds of older and degenerating corpora lutea. In the nursing animals, on the other hand, we only observe older corpora lutea which evidently originated as the result of

[1] These investigations were carried out during the year 1918 and in the first part of 1919. After the return of Doctor Kuramitsu to Japan some additional experiments were made in 1919. At the last meeting of the Association of Anatomists, J. A. Long and H. M. Evans presented the results of very extensive studies on the sexual cycle in the rat. In the course of these investigations they concluded that during lactation ovulation is suspended in the rat, and that the corpus luteum of lactation is intermediate in size between the corpus luteum of pregnancy and the ordinary corpus luteum. It furthermore differs from the other corpora lutea through the presence of diffusely distributed granules in the lutein cells. As far as the rat is concerned, the results of Evans and Long and ourselves are in agreement.

ovulation which usually occurs spontaneously within the first twenty-four hours following labor. In addition we found retrogressing corpora lutea. This typical difference between the corpora lutea of nursing and non-nursing rats, we noticed at as early a period as seven days and as late as four weeks after labor. Five weeks after labor we likewise found a greater number of young corpora lutea in the non-nursing rat; again the ovaries were larger in this animal, but in this case ovulation had not taken place quite so recently as in the large majority of the other ovaries of the non-nursing animals. In addition we usually observed some further differences between the ovaries of the non-nursing and the nursing animals. In the former the follicles usually reached a larger size than in the nursing rats. Occasionally we found relatively large follicles in the nursing animals; in this case, however, the large well-preserved follicles are generally more numerous in the non-nursing rats. Furthermore in these animals the center of the ovary is usually more succulent, the lymph vessels seem to be more dilated, and that part of the so-called interstitial gland which is situated around the vessels in the central part of the ovaries is in most cases larger than in the nursing animals. In the non-nursing rat these interstitial cells may assume an epithelioid character. We merely state at present these latter differences without necessarily assuming a causal relationship between the character of the follicles and interstitial gland and lactation or absence of lactation, although we consider such a connection as probable.

According to Long and Evans (1) ovulation occurs in the rat on the average every four or five days. Our findings in the non-nursing rats are in agreement with this conclusion. In the majority of these animals we find freshly formed corpora lutea. In this case ovulation takes place at short intervals. In the nursing animal, on the other hand, lactation prevents ovulation. As to the mechanism underlying this inhibiting effect of lactation, we have to consider two possible explanations. *a*, We found indications that the process of nursing is unfavorable for the full and rapid growth of the follicles. In the ovaries which we observed they seemed to degenerate at a somewhat earlier stage of development in the nursing rats. This in turn may be due to better circulatory conditions in the ovaries of the non-nursing animals which leads to a more succulent condition of the stroma and in a number of cases to a larger size of the central part of the interstitial tissue. While, as we stated, these findings may be due to a coincidence, it is more probable that a causal relation exists between lactation or absence of lactation

and the general condition of the ovaries. *b*, In the guinea pig we have shown experimentally that the corpus luteum prevents ovulation during the ordinary cycles as well as during pregnancy. Possibly in the rat the ordinary corpus luteum does not possess the inhibiting function or rapidly loses it. However that may be, the corpus luteum of pregnancy which is better developed than the ordinary corpus luteum does possess the inhibiting function even in the rat, as the following observation shows. Rat b (II series) had again become pregnant soon after labor. The young were at once removed from the mother. Examination three weeks after labor revealed two living and one dead embryo. The ovaries contain large, well-preserved corpora lutea, larger than the corpora lutea of a lactating, but not pregnant rat. In addition we find retrogressing vacuolar corpora lutea with some lymphocytic infiltration; connective tissue and lymphocytes are substituted for some of the lutein tissue. Atretic yellow vacuolar bodies are also found. There are well preserved follicles of various sizes as well as follicles in granulosa degeneration. Much small-celled interstitial tissue is present. In this case new ovulations had not taken place, despite the fact that the animal did not nurse her young. The corpora lutea which we observe are the large corpora lutea of pregnancy. Evidently here as in other animals the corpus luteum of pregnancy prevented ovulation. There is therefore some reason for assuming that the corpus luteum of lactation may in the rat have a function similar to that of the corpus luteum of pregnancy although perhaps less marked.

We cite as an example of the differences found in the ovaries of nursing and non-nursing rats our findings in rats 19 and 18.

Rat 19 (I series): Four weeks after labor; the rat nursed her young. Large corporea lutea; many nuclei of the lutein cells are well preserved, vesicular. Some nuclei, however, are deformed. At places toward the center of the corpora lutea we find areas of vacuolization. In such a central vacuolar area can be seen polynuclear leucocytes which occasionally penetrate into the vacuolar cells. In the peripheral part of this area we find collections of lymphocytes. In addition we find small shrunken corpora lutea in which the capillaries are relatively prominent and the lutein cells small. Some atretic vacuolar bodies (remnants of degenerated corpora lutea) are also seen. There are well-preserved follicles of small and medium size and a number of follicles in granulosa degeneration. Phagocytes take up the degenerating granulosa and in turn degenerate again. We find many atretic follicles with small-celled theca interna.

Rat 18 (I series): Four weeks after labor. Animal did not nurse her young. In this animal the ovaries are much larger and the number of more or less well-preserved corpora lutea is much greater. A number of follicles have just ruptured and vessels are growing into the granulosa. The cavities of the new corpora lutea are as yet incompletely organized. Other corpora lutea are vacuolar. The great majority of follicles are well preserved; especially prominent are well-preserved follicles of large size. In this respect these ovaries differ from those of rat 19; on the other hand the number of quite atretic follicles is smaller in this animal than in no. 19. A part of the stroma is succulent, edematous; and there are areas where the interstitial cells are large. Lymphocytes occur in this interstitial tissue.

The influence of lactation on ovulation in the guinea pig. In our former studies of the cyclic changes in the ovary of the guinea pig (2, 4, 6) we have occasionally examined ovaries in the period following labor without noticing a marked influence of lactation on ovulation and the sexual cycle. Similarly O. Ishii (5) has noticed the occurrence of ovulation in the period following labor. In view of the findings in the rat, however, it was desirable to make a special study of ovulation during the period on lactation in the guinea pig. We compared in each case at various periods following labor the ovaries of guinea pigs which nursed their young as well as the ovaries of guinea pigs from which the young had been removed directly after labor. Soon after labor a spontaneous ovulation occurs, as we found in confirmation of our previous observations. Furthermore our previous studies had shown that in the large majority of cases a spontaneous ovulation occurs in the guinea pig every fifteen to eighteen days. The observations of O. Ishii and Stockard and Papanicolaou (7) confirm these findings. In this respect the rat differs from the guinea pig. Long and Evans have shown that in the former ovulation is much more frequent. We should accordingly not expect an ovulation in the guinea pig within the first two weeks following labor, but after that period ovulation should occur in both the nursing and the non-nursing guinea pigs. This is what actually occurs. We may conclude from our experiments that in the nursing guinea pig ovulation occurs as well as in the non-nursing animal and furthermore that the cyclic changes which we described previously in the ovary of the guinea pig are the same in both. Our studies have shown furthermore that such cyclic changes as we found in the ovary of the guinea pig do not occur in the ovary of the rat. In the latter ovulation is not followed by such a far-going transformation in the condition of the

whole ovary as in the guinea pig. Whether slight quantitative differences in the frequency of ovulation exist in the nursing and the non-nursing guinea pig, we cannot yet state definitely, but there is so far no indication of a noticeable delay in the nursing animal.

Within the first two weeks following labor we found a greater number of well-preserved large follicles in the non-nursing than in the nursing guinea pig. Similarly after two weeks there were mature follicles in the ovaries of the non-nursing, but not in those of the nursing animal. Possibly these differences are due to a coincidence; however, they correspond to analogous findings in the rat. Three, four and five weeks after labor we found in the nursing animal corpora lutea which had been formed during the period of lactation; they corresponded to similar corpora lutea in the non-nursing animal. In the ovaries examined three weeks after labor the microscopic examination of ovaries and uterus showed that a new ovulation had occurred eighteen to nineteen days after labor in the nursing and seventeen to eighteen days after labor in the non-nursing guinea pig. The specimens examined four weeks after labor showed the occurrence of a new ovulation about fifteen to eighteen days following labor in the nursing and approximately twenty-three days following labor in the non-nursing guinea pig. In animals examined five weeks after labor ovulation had likewise occurred, namely, about thirty-two to thirty-three days and probably sixteen to eighteen days following labor in the nursing guinea pig and twenty-nine to thirty days following labor in the non-nursing animal. We may cite more in detail our findings in the guinea pigs examined three weeks after labor.

a. In the nursing guinea pig we find young corpora lutea. A small amount of connective tissue has grown into the periphery of the central cavity in these corpora lutea. Atretic vacuolar corpora lutea originating from the ovulation directly following labor. Some well-preserved follicles of small and small to medium size. Larger follicles without granulosa; connective tissue begins to grow into these cavities. By far the greater part of the ovaries consists of completely atretic follicles. The shrunken thecae internae either surround directly the zona pellucida or they surround loose fibrous tissue which fills the cavities of the follicles. A new ovulation had occurred about two and a half days previous to examination.

b. In the non-nursing guinea pig we also find newly formed corpora lutea, with central cavities which have been reduced in size through ingrowing connective tissue to a greater extent than in the nursing ani-

mal. In the non-nursing guinea pig the corpora lutea are somewhat larger. Pale, retrogressing, vacuolar corpora lutea with thick vessels. They formed as the result of the ovulation immediately following labor. Polynuclear leucocytes collect around these retrogressing corpora lutea. In addition there are pale remnants of the corpora lutea of pregnancy. Well-preserved follicles of small and small to medium size. Follicles in connective tissue atresia, especially many follicles in the last stage of connective tissue atresia. In this animal an ovulation had occurred about three to four days previous to examination. The young corpora lutea are here one-half to one and one-half days older than in the nursing animal. In the non-nursing animal the well-preserved follicles are a little larger than in the nursing guinea pig.

The conditions in both guinea pigs are therefore similar, but in the non-nursing animal ovulation occurred at a slightly earlier date.

We may therefore conclude that there exists a marked difference between the behavior of the rat and the guinea pig as to the influence of lactation on ovulation and the sexual cycle. In the former ovulation is suspended during the period of nursing; in the latter it continues to take place. Accordingly we notice that in the lactating guinea pig degenerative changes set in in the corpora lutea formed during the period following labor at approximately the same time as in the non-nursing guinea pig. As we have shown previously the commencing retrogression in the corpus luteum of the previous cycle is prerequisite to a new ovulation in the guinea pig. It is not improbable that in the rat the corpus luteum remains functionally active during a longer period than in the guinea pig and that there exists a further-going functional difference between the ordinary corpora lutea and those of lactation in the rat than in the guinea pig.

CONCLUSIONS

1. There exists a marked difference between the behavior of the rat and the guinea pig as to the influence of lactation on ovulation and the sexual cycle. In the former ovulation is suspended during the period of nursing, while in the latter it continues to take place.

2. In the guinea pig ovulation is followed in the nursing and in the non-nursing individual by the same cyclic transformation of the ovary. In the ovary of the rat ovulation is not followed by cyclic changes comparable to those of the guinea pig.

3. It may be suggested as a possible explanation of the difference found between the behavior of the rat and guinea pig, that in the rat the corpus luteum of lactation is functioning during a longer period of time than in the guinea pig.

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REVERSAL OF DEPRESSOR ACTION OF SMALL DOSES OF ADRENALIN

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Cannon and Lyman (1) demonstrated that under different circumstances small doses of adrenalin might have opposed effects on the blood pressure. Thus they found that the depressor action of a small dose of adrenalin injected into an animal with normal blood pressure could be converted into a pressor action when the blood pressure had been lowered as by pithing. Hartman (2) later showed that the effect of small doses of adrenalin administered intravenously was to cause dilatation of the limb vessels and constriction of the vessels of the splanchnic area. Hoskins, Gunning and Berry (3) found that in small doses, adrenalin given by intravenous injection caused dilatation of the muscle vessels and constriction of those in the cutaneous area. Dale (4) was of the opinion that adrenalin dilatation was due to the stimulation of vaso-dilator nerve endings in the vessel wall. Gruber (5) was unable to obtain vaso-dilatation in limb vessels the nerves to which had just been severed. He concluded that lack of vaso-dilator action after small doses of adrenalin in denervated limbs was due to loss of tonicity. He also suggested that the vaso-dilatation observed by Hartman and Fraser (6), and accredited by them to direct stimulation of a central vasodilator center by adrenalin, was due to depression of the vaso-constrictor center as a result of increased blood pressure. Snyder and Andrus (7) obtained reversal of the effect of adrenalin on the isolated heart of the terrapin by alteration of the hydrogen ion concentration of the perfusate. Thus they found that adrenalin, while augmenting the tonus evoked by a Ringer solution of 7.8 pH index, still further diminished the atony evoked by a Ringer solution of pH 7.0. Synder and Campbell (8) have recently reported reversal of the constrictor effect of adrenalin on the perfused vessels of the frog following increase in the hydrogen ion concentration of the perfusate while

the constrictor effect was even increased by decreased hydrogen ion concentration of the perfusing fluid. Collip (9) has obtained antagonism of the depressor effect of small doses of adrenalin and augmentation of the pressor effect of larger doses by the intravenous administration of tissue extract. He suggested that this effect was due to depression of the peripheral vaso-dilator mechanism by some constituent or constituents of tissue extract. In the course of a further study of the antagonistic effect of tissue extracts upon the depressor action of minute doses of adrenalin given by the intravenous route, other points of an interesting character became manifest. It is of these latter that this paper will deal.

Methods. Dogs were used. The routine preparation consisted of the insertion of a tracheal cannula which served for administration of the anesthetic by the Woulff bottle method, and of the cannularization of the left carotid artery and the left external jugular vein. Blood pressure was recorded by use of the mercury manometer. Injections were made directly into a rubber tube connected to the jugular cannula and the injected fluid was flushed into the vein by means of a few cubic centimeters of physiological saline supplied from a burette. As a rule both vagi were cut.

Results: Effect of altering the depth of anesthesia. It was noted that reversal of the depressor action of a small dose of adrenalin, given by intravenous injection, could be effected by increasing the anesthetic. The depressor effect again returned when light anesthesia was resumed. (Fig. 1, *A* and *B*.) This reversal is brought about very quickly, particularly when ether is used as an anesthetic. The same results were manifested when chloroform was used, but with chloroform the conversion of a pressor effect with deep anesthesia into a depressor effect with light anesthesia is more slowly accomplished. This is probably due to the fact that chloroform is a much more potent anesthetic than ether. The graphic record of an experiment in which ether was the anesthetic used is shown in figure 1. It is remarkable with what promptness the reversal of the effect of a small dose of adrenalin is manifested. This is equally true for both types of the phenomenon. When the animal is receiving no ether the effect of a small dose of adrenalin is a fall in blood pressure, while the same dose given almost immediately after full ether has been allowed causes a rise in blood pressure. Allowing no ether again restores the depressor action and thus the reversal can be accomplished over and over again. The promptness with which the phenomenon can be elicited suggests that

probably some factor other than the degree of concentration of the anesthetic in the blood is active. It is possible, however, that the slightest change in the concentration of the anesthetic in the blood is in itself sufficient to elicit the effect. That the effect is not due simply to a lowering or a raising of the blood pressure as in the case of the experiments of Cannon and Lyman (1) is shown by the observation that a reversal may be noted at equal levels of blood pressure (fig. 1).

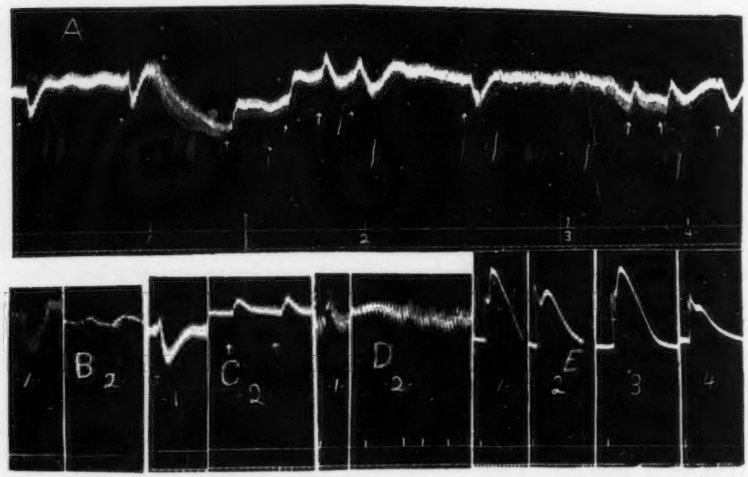

Fig. 1. *A:* Dog ♂ 14 kilo vagi cut—very light ether anesthesia. At arrow marks 1 cc. of 1:400,000 adrenalin injected into left jugular vein. Between *1* and *2*, and *3* and *4*, full ether was administered. Before *1*, between *2* and *3*, and after *4* no ether was administered. Time in 10 second intervals. Base line above time tracing.

B: Dog ♂ 16 kilo vagi cut—atropinized.

1. Effect of 1 cc. of 1:100,000 adrenalin, light ether anesthesia.

2. Effect of 1 cc. of 1:100,000 adrenalin, deep ether anesthesia. Time 2 second intervals.

C: Dog ♂ 14 kilo. Light ether anesthesia, vagi cut, atropinized. 1 cc. of 1:400,000 adrenalin injected at arrow marks. Between *1* and *2*, 40 cc. of 20 per cent Na_2CO_3 injected. Time 10 second intervals.

D: Same animal as above; 1 cc. of 1:400,000 adrenalin injected at signal marks on base line. Between *1* and *2*, 50 cc. of 7 per cent NaH_2PO_4 injected. Time 10 second intervals.

E: Dog ♂ 12 kilo. Vagi cut, atropinized. Effect of ½ cc. of 1:12,000 adrenalin. *1.* Control. *2.* Four minutes after 10 cc. 10 per cent NaH_2PO_4 intravenous. *3.* Five minutes after 15 cc. 20 per cent Na_2CO_3 intravenous. *4.* Five minutes after 50 cc. 10 per cent NaH_2PO_4 intravenous. Time 10 second intervals.

Effect of altering the C_H *of the blood.* It was also found that reversal of the depressor action of a small dose of adrenalin could be obtained by suddenly increasing the alkalinity of the blood by the administration of a fairly large dose of sodium carbonate, the anesthesia being constant throughout (fig. 1, *C*). The subsequent increase in the C_H of the blood following the intravenous injection of acid sodium phosphate antagonized the pressor effect of adrenalin (fig. 1, *D*). This result may be obtained repeatedly in the same animal. Also the pressor response to a larger dose of adrenalin is augmented by an increase in the alkalinity of the blood and is decreased by a decrease in the alkalinity (fig. 1, *E*).

Non-effect of atropine. It was found that the atropinized animal responded in the same manner as regards the reversal of the adrenalin effect to altered C_H and to altered depth of anesthesia as the unatropinized animal. It was also noted that atropine did not counteract the antagonistic effect of tissue extract upon the depressor action of small doses of adrenalin.

DISCUSSION

These observations on the effect of alteration in the reaction of the blood upon the response to adrenalin given by intravenous injection into the intact animal under light but constant ether anesthesia are in agreement with the findings of Snyder and Andrus (7) and Snyder and Campbell (8) in respect to the reversal of the adrenalin effect on the heart of the terrapin and the perfused vessels of the frog with altered C_H of the perfusing fluid. The former reaction was explained on the basis that the hydrogen ion concentration determines the tonus irritability of the muscle substance only, whereas the drug acts upon the myoneural junctions of the sympathetic nerve fibers. If the tonus of the muscle substance is positive then epinephrin will increase it; if the tonus in the muscle is negative epinephrin will decrease it still further. As the blood pressure in the experiments reported in this paper was practically at the same level on several occasions when each of the two types of response to adrenalin injection was obtained it is difficult to see how the above theory could be applied to explain the phenomenon observed in these instances. The altered C_H of the blood is undoubtedly the basis for the modification of the reaction of the blood pressure of the intact animal to adrenalin in these instances. Whether there is selective action on some special structure or any special set of vessels the writer is not prepared to state.

The reversal of the circulatory response to small doses of adrenalin caused by alteration of the intensity of anesthesia is very likely due to

an entirely different cause than the similar reversal noted, following altered blood C_H.

The observation of Dale (10) that shock following the injection of histamine into cats is elicited with a very much smaller dose of the drug if the animal has been anesthetized with ether is of interest in this connection.

A remarkable similarity therefore exists between the effect of the decreasing of the C_H of the blood, the increasing of the depth of anesthesia, the intravenous injection of tissue extract, and the experimental decrease in the tonus of the arterioles, as regards the resultant action on the circulatory response of the intact anesthetized animal to small doses of adrenalin.

SUMMARY

1. The depressor effect of a small dose of adrenalin in an animal under light ether or chloroform anesthesia may be converted into a pure pressor effect by increasing the anesthetic. Decreasing the anesthetic restores the depressor effect.

2. The depressor effect of a small dose of adrenalin in an animal under light but constant anesthesia may be converted into a pressor effect by decreasing the blood C_H by means of intravenous administration of sodium carbonate.

3. The pressor effect of a moderate dose of adrenalin may be increased by decreasing the blood C_H.

4. The pressor effect of a moderate dose of adrenalin may be decreased by raising the C_H of the blood as by intravenous injection of acid sodium phosphate.

5. Atropine does not antagonize the above reactions nor does it effect the antagonism of the depressor action of small doses of adrenalin by tissues extracts.

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STUDIES IN NUTRITION

V. The Nutritive Value of Soy Bean Flour as a Supplement to Wheat Flour

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It has been shown in a previous publication (1) from this laboratory that peanut flour is an efficient supplement to wheat flour. We have made a similar series of experiments with soy bean flour and found it to be equally efficient.

Soy beans have long been used in the human dietary by the peoples of the Orient. Because of their low starch content, soy beans also have been used to some extent as a diabetic food in this country. The demand for oil, both for industrial and edible purposes, has resulted in large importations of this oil-bearing seed as well as its more extensive cultivation in this country.

The press cake, which is a by-product remaining after the oil has been expressed from soy beans, has hitherto been employed mainly as a stock feed or fertilizer. A flour suitable for human food can be obtained by grinding this press cake. Such a flour contains about 7 per cent of fat and 43 per cent of protein ($N \times 5.7$).

The food value of soy beans has been shown by the nutrition experiments of Osborne and Mendel (2), (3), and Daniels and Nichols (4). Holmes (5) has demonstrated by means of digestibility experiments with human subjects that the proteins, fats, and carbohydrates of soy bean flour are efficiently utilized. The addition of 15 to 25 per cent of soy bean flour to wheat flour in making bread produces a palatable and very nutritious loaf. The mixture of proteins in this bread is more efficient than the proteins of wheat flour alone. Such a bread also contains an abundance of water-soluble vitamines.

Bread used in experiments. Breads were made from mixtures of wheat[1] and soy bean flour. Those containing 25 parts of soy bean flour and 75 parts of wheat flour will be designated as 25 per cent soy bean bread, while those made from 15 parts of soy bean flour and 85 parts of wheat flour will be called 15 per cent soy bean bread.

The ingredients used in each loaf[2] were:

	25 PER CENT SOY BEAN BREAD	15 PER CENT SOY BEAN BREAD
	gm.	*gm.*
Wheat flour	300	340
Soy bean flour	100	60
Compressed yeast	10	10
Granulated sugar	10	10
Sodium chloride	6	6
Water	228	228

After baking, the bread was sliced, dried at 60°C., and ground to a meal, which was used for preparing the diets.

Growth obtained on 25 per cent soy bean bread. A diet containing 15.8 per cent of protein was prepared from bread made with flour A. A similar diet was prepared from bread made with flour B. These diets contained 80 parts of dried bread, 16 parts of butter fat and 4 parts of an inorganic salt mixture.[3] The rats fed these diets grew normally. This is in contrast to the poorer growth obtained when wheat bread (1) furnished the sole source of protein in an otherwise adequate diet. In the latter experiment the rats grew at only one-third to two-thirds of the normal rate. The composition of the soy bean bread diet and growth curves are shown on chart 1.

In order to ascertain if the lower rate of growth on the wheat bread diet controls was due to its lower protein content, a similar diet was prepared from the 25 per cent soy bean bread by replacing a part of

[1] Two types of wheat flour were used to prepare the breads. One was a "war flour" containing about 74 per cent of the wheat kernel; the other, which was milled for another project, was approximately a "straight flour." The former flour we have designated as flour A, and the latter as flour B.

[2] Ten such loaves were baked each time. The salt and sugar were dissolved in the water and the solution was mixed with the yeast. This suspension was mixed with the flour and the resulting dough handled in the usual manner.

[3] For composition of salt mixture, see Osborne, T. B., and Mendel, L. B., Journ. Biol. Chem.. 1917, xxxii, 374.

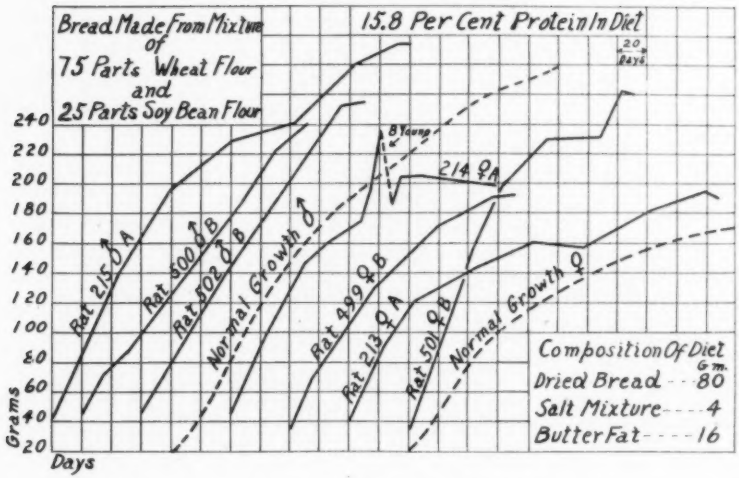
Bread Made From Mixture of 75 Parts Wheat Flour and 25 Parts Soy Bean Flour
15.8 Per Cent Protein In Diet
20 Days
8 Young Rats
214 ♀ A
Rat 215 ♂ A
Rat 500 ♂ B
Rat 502 ♂ B
Normal Growth ♂
Rat 429 ♀ B
Rat 213 ♀ A
Rat 501 ♀ B
Normal Growth ♀
Composition Of Diet
Gm.
Dried Bread - - 80
Salt Mixture - - - 4
Butter Fat - - - - 16
Grams
Days

Chart 1

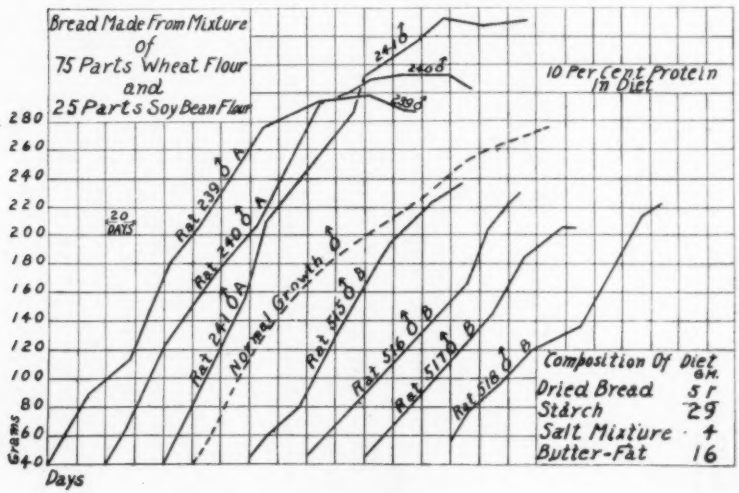

Chart 2

TABLE 1

Gain of body weight per gram of ingested protein furnished by 15 and 25 per cent soy bean bread and wheat bread

SOURCE OF PROTEIN	PROTEIN IN FOOD			RAT	INITIAL BODY WEIGHT	GAIN IN 4 WEEKS	GAIN IN 10 WEEKS	4-WEEK PERIOD					10-WEEK PERIOD				
								Total intake		Intake per gram of gain			Total intake		Intake per gram of gain		
	From wheat flour	From soy bean flour	Total protein in diet					Food	Protein	Food	Protein	Gain per gram of protein	Food	Protein	Food	Protein	Gain per gram of protein
	per cent	*per cent*	*per cent*		*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
25 per cent soy bean bread "war flour"	7.1	8.7	15.8	213 ♀	42	52	95	178	28.1	3.42	0.54	1.85	517	81.7	5.44	0.86	1.16
				214 ♀	45	61	123	223	35.2	3.66	0.58	1.73	661	104.4	5.37	0.85	1.18
				215 ♂	43	62	140	189	29.9	3.05	0.48	2.07	591	93.4	4.22	0.67	1.50
Average										3.38	0.53	*1.88*			5.01	0.79	*1.28*
25 per cent soy bean bread "war flour"	4.5	5.5	10.0	239 ♂	40	50	110	210	21.0	4.20	0.42	2.38	583	58.3	5.30	0.53	1.89
				240 ♂	40	53	122	227	22.7	4.28	0.43	2.34	635	63.5	5.21	0.52	1.92
				241 ♂	42	54	169	256	25.6	4.74	0.47	2.11	760	76.0	4.50	0.45	2.22
Average										4.41	0.44	*2.28*			5.00	0.50	*2.01*
25 per cent soy bean bread approximately a "straight flour"	7.2	8.6	15.8	499 ♀	36	58	115	213	33.7	3.67	0.58	1.72	523	82.6	4.55	0.72	1.39
				500 ♂	44	42	96	216	34.1	5.14	0.81	1.23	506	80.0	5.27	0.83	1.20
				501 ♀	36	78	168	279	44.1	3.58	0.57	1.77	725	114.5	4.32	0.68	1.47
				502 ♂	46	44	119	183	28.9	4.16	0.66	1.52	515	81.4	4.33	0.68	1.46
Average										4.14	0.66	*1.56*			4.62	0.73	*1.38*

25 per cent soy bean bread approximately a "straight flour"	4.6	5.4	10.0	515♂	44	30	98	206	20.6	6.87	0.69	1.46	620	62.0	6.33	0.63	1.58
				516♂	45	27	67	224	22.4	8.30	0.83	1.21	555	55.5	8.28	0.83	1.21
				517♂	44	31	71	161	16.1	5.19	0.52	1.93	461	46.1	6.49	0.65	1.54
				518♂	58	34	66	200	20.0	5.88	0.59	1.70	524	52.4	7.94	0.79	1.26
Average										6.56	0.66	*1.58*			7.26	0.73	*1.40*
15 per cent soy bean bread "war flour"	8.0	5.3	13.3	321♂	32	48	115	186	24.7	3.88	0.52	1.94	523	69.6	4.55	0.61	1.65
				322♀	36	37	81	150	20.0	4.05	0.54	1.85	434	57.7	5.36	0.71	1.40
				323♂	35	39	108	167	22.2	4.28	0.57	1.76	500	66.5	4.63	0.62	1.62
Average										4.14	0.54	*1.85*			4.84	0.65	*1.56*
*Wheat bread "war flour"	10.0	0	10.0														
Average										15.9	1.59	*0.64*			10.8	1.08	*0.96*
*Wheat bread approximately a "straight flour"	10.0	0	10.0														
Average										9.65	0.96	*1.04*			10.1	1.01	*0.99*

* Johns, C. O., and Finks, A. J., *Journ. Biol. Chem.*, 1920, xlii, 569.

the bread meal with starch. The protein content of this diet was the same as the wheat bread control diet, namely 10 per cent. Except for a slight lag in the initial rate of growth all of these rats attained full normal size. From this it is apparent that the addition of soy bean flour to wheat flour in making bread produces a mixture of proteins which is more efficient than the proteins in wheat bread alone. The results and composition of the diet are recorded on chart 2.

Growth obtained on 15 per cent soy bean bread. Normal growth was obtained on a diet made with this bread. The protein content of this diet was 13.3 per cent. The growth curves and composition of the diet are shown on chart 3.

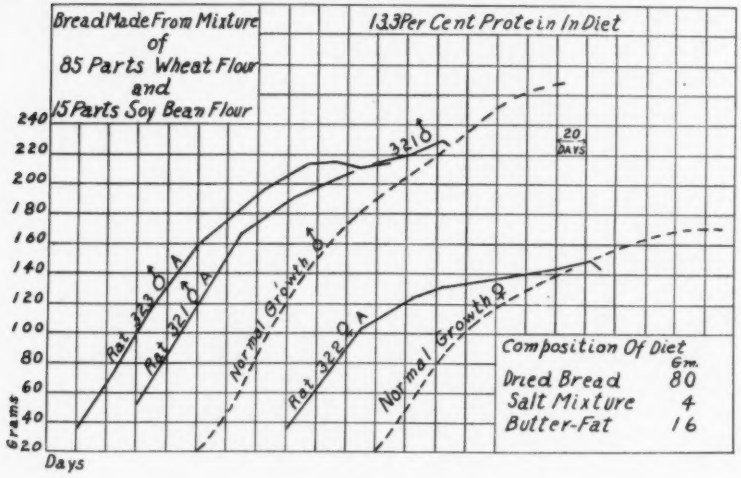

Chart 3

Efficiency of the proteins in the different breads. In order to compare the efficiency of the various diets, the food intakes were recorded. We have followed the method outlined by Osborne, Mendel and Ferry (6). The index numbers showing the ratio of the gain in weight to grams of protein ingested are shown in table 1.

It is apparent from these data that the mixture of soy bean and wheat proteins is much better utilized than the wheat proteins alone. Comparison of the index numbers obtained show that for the same amount of food consumed, the soy bean bread diets are about two or three times as efficient as the wheat bread diets.

SUMMARY

1. Bread made with a mixture of 25 parts of soy bean flour and 75 parts of wheat flour contained a protein mixture and water soluble vitamines adequate for normal growth. A similar bread containing 15 parts of soy bean flour and 85 parts of wheat flour likewise furnished adequate proteins and water-soluble vitamines for normal growth.

2. These mixtures of the soy bean and wheat proteins were found two or three times more efficient than the proteins from wheat alone.

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IV. FURTHER EXPERIMENTS ON THE EFFECT OF WARMING AND COOLING THE SINO-AURICULAR NODE IN THE MAMMALIAN HEART—DEPTH OF ANESTHESIA; TACHYCARDIA; FLUTTER; SINO-AURICULAR HEART BLOCK

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This paper is a continuation of our studies (1), (2), (3) on the response of the heart when the sino-auricular node is warmed or cooled. Especial reference is here made to *a*, the effect of depth of ether anesthesia; *b*, to continuous periods of warming; *c*, to the production of paroxysmal tachycardia; *d*, auricular flutter; and *e*, sino-auricular heart block.

Method. The operative procedure on dogs and cats, mainly the former, was as follows: Ether was administered, a tracheal cannula inserted and the thorax was opened under artificial respiration (Gesell-Erlanger apparatus), after preliminary ligation of the internal mammary arteries. The phrenic nerves were cut and the pericardium sewed back. A ligature inserted in the adipose tissue of the A–V groove was used to turn the heart gently toward the left, thereby giving adequate exposure of the region of the sino-auricular node. The exposure was similar to that shown by Schlomovitz and Chase (3, p. 616). Records were taken on a modified Hürthle electrical kymographion by receiving tambours connected by air transmission to recording tambours. The receiving tambours responded to the movements of the right auricle and right ventricle. Time was recorded by a Jacquet chronometer and a 0.02 second electromagnetic tuning fork. The same type of thermode was used as in figure 1 (3). The thermode tip was of copper, 0.002 of an inch thick, permitting better temperature conduction. An ethyl chloride spray was also used to cool the node. A critical analysis of the temperature method can be found in (3). The procedure proper to each series was then instituted.

SERIES I. THE EFFECT OF DEPTH OF ETHER ANESTHESIA

The logical procedure following the anatomical discovery of the specialized tissue of the mammalian heart, and the proof of its physiological importance, is a determination of the tissue's reaction to pharmacological as well as chemical agents. This has been done to a small degree. Ethyl chloride (4), (5), nicotine (4), (6), (7), atropine (4), muscarine (4), curare (4), cocaine (8), adrenalin (9), sodium salicylate, bile, bile salts, chloroform, ethyl alcohol, caffein sodiobenzoate and theobromin (10) have been applied directly to the sino-auricular node. Isolated strips of the cat's heart, including a strip containing the sino-auricular node, have been bathed in solutions containing pilocarpine, physostigmine, atropine, nicotine, adrenalin, curara, apocodeine, ergotoxine and pituitary extract. The point of origin of the heart beat has been studied after the injection of potassium chloride (5). We are not aware of any study dealing with varied concentrations of ether in the blood stream coupled with simultaneous local treatment of the node.

It is well known that continued administration of ether produces a slowing, weakening and dilatation of the heart. It is evident that the function of automaticity as well as the functions of contractility and conductivity may be affected, but by the methods heretofore used such a fact could not be definitely asserted. It has been shown (11) that one of two things may happen when there is a physiological decrease in cardiac rate beyond what may be termed the normal variations. The pacemaker may shift either to a lower point in its original site—the sino-auricular node—or else it leaves its normal anatomical locus and is then found lower down in the specialized tissue of the heart. Aside from physiological variations in the irritability, we may have the irritability modified by outside influences. Two other possibilities may arise, third, the irritability of the cardiac tissue proper, the muscle, may be affected so that it responds less readily to the normal stimulus initiation, which may result in some form of heart block; and fourth, there may be a simultaneous and coördinate decrease in the functional manifestations of both specialized tissue and cardiac tissue proper, as for instance when the whole heart is cooled. The third possibility is illustrated by the reaction of skeletal muscle in deep ether narcosis. Biederman states that the local capacity of reaction in muscle seems not to be perceptibly affected by narcosis, as far as may be judged from the galvanometric changes visible (12). This is probably true of the sino-auricular node in deep ether narcosis.

The data have been arranged in the following groups, according to the effects produced on the S-A node during:

A. Light ether anesthesia: experiments 1 to 11.
  1. Response to short intermittent periods of warming.
    a. Without atropine.
    b. With atropine.
  2. Response to long continuous periods of warming.
B. Long continued ether anesthesia: Experiment 12.
  1. Response throughout a long period (more than 8 hours) under light and deep anesthesia.
C. Deep ether anesthesia; experiments 12 to 17.
  1. Response in a thoroughly anesthetized animal (heart weakened and slowed by ether). Warming and cooling effects are noted.
  2. Response in such an animal after the administration of atropine.

A. *Effects of light ether anesthesia:* Studies in the responsiveness of the sino-auricular node by warming it for rather short periods in animals under general ether anesthesia have been carried on by a great many investigators—MacWilliam, Adam, Hering and Koch, Jaeger, Flack, Ganter and Zahn, Brandenburg and Hoffman, Zahn, Schlomovitz and Chase (*q.v.* for bibliography). These have all shown that there is an immediate and rapid increase in rate, and at times marked tachycardia. In our first experiments the above work was repeated, and in addition attempts were made to increase the temperature to a point where its transitory application would produce a continued tachycardia. Such attempts were invariably ineffective. Heart rates of 225 to 348 per minute, marked increases over the normal, were elicited by temporary periods of warming in animals lightly anesthetized. Upon removal of the thermode, there was either an immediate return to normal, or else a temporary decrease which was less than normal and eventually a return to normal. The latter is illustrated by record 7 of experiment 3 (fig. 1).

This figure also shows the occurrence on warming of one long cardiac cycle rate of 113 per minute, after five short beats with a rate of approximately 240 per minute. This abnormality will be discussed below under sino-auricular block. The heart had a normal rate of about 162 per minute. The thermode was warmed to 57°C. Marked lengthening of the beat as a rule appeared only when the temperature was high, and during a short period of warming. Occasionally a rate a

little greater than normal prevailed for several seconds after the warming.

In addition to the data obtained above on non-atropinized animals, a few experiments were done in which the effects of warming were noted before and after atropine administration. Experiment 9 is typical. A pup, 1.8 kilograms in weight, had a normal rate of 126 to 143 for an entire hour. Warming the sino-auricular node with the thermode at 42°C. produced increases of 214 to 246 beats per minute. These were increases of 150 to 200 per cent over the normal. These increases occurred before and after the administration of 2 mgm. of atropine sulphate. In other words, atropine administration did not alter the reaction of the node.

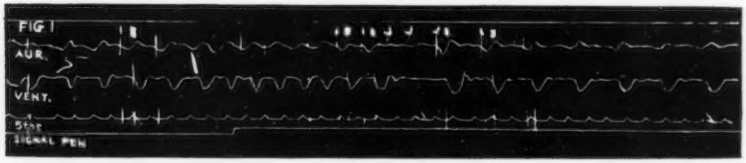

Fig. 1. Suspension tracings of right auricle and right ventricle in the dog. The down stroke indicates systole. Warming the sino-auricular node produced a temporary tachycardia; increase from 162 to 240 per minute. This was followed by one long beat, after which all the beats were lengthened somewhat. Finally the rate returned to normal. The narrow white line represents systolic onset of the auricle. The broad white line or square represents systolic onset of the ventricle.

The effect of cooling the sino-auricular node was noted in experiment 15. A 19 kilogram dog was given one-sixth grain of atropine sulphate. The normal rate of 136 was decreased to 122 beats per minute with a temperature of 7°C. An ethyl chloride spray decreased the rate to 118 and finally to 107. This occurred under light anesthesia.

The response of the sino-auricular node to continuous periods of warming of comparatively long duration was entirely different from the above. Upon application of the thermode to the node there was the usual immediate increase in rate but after intervals of a minute or more the rate would decrease gradually to about normal, and in some cases to less than normal. With the decreased rate, there would often occur a decrease in the A_sV_s interval. Our method does not permit us to say whether A–V rhythm prevailed except where we cooled

the sino-auricular node. In experiment 1 the normal rate of 146 per minute was increased at once to 196 on warming, and decreased to 164 after 5 minutes warming (nos. 34, 36, 37). In experiment 4 the normal rate of 168 was increased to 294 by a temperature of 46.5°C. with

TABLE 1

Illustrates the original increase produced by warming and progressive decrease in cardiac rate and A_sV_s interval by continuous warming. The rate drops below normal on removal of the thermode

MINUTES	PROCEDURE	A_sV_s	RATE PER MINUTE	PER CENT OF NORMAL
		seconds		
0	Normal rate	0.09	143	100
	42°C. thermode on node	0.10	214	150
1 min. after	42°C. thermode on node	0.10	198	139
2 min. after	42°C. thermode on node	0.10	214	150
5 min. after	42°C. thermode on node	0.10	214	150
6 min. after	42°C. thermode on node	0.10	198	139
7 min. after	42°C. thermode on node	0.10	204	143
8 min. after	42°C. thermode on node	0.09	198	130
9 min. after	42°C. thermode on node	0.09	204	150
10 min. after	42°C. tehrmode on node	0.09	186	130
11 min. after	42°C. thermode on node	0.08	186	130
12 min. after	42°C. thermode on node	0.08	162	113
13 min. after	42°C. thermode on node	0.06	150	105
14 min. after	42°C. thermode on node	0.09	162	113
15 min. after	42°C. thermode on node	0.11	162	113
	Thermode removed	0.09	120	84

An abrupt drop in rate below normal on removal of the thermode is also shown in table 2, taken from experiment 7. It will be noticed that the A_sV_s interval varies slightly throughout.

TABLE 2

MINUTES	PROCEDURE	A_sV_s	RATE PER MINUTE	PER CENT OF NORMAL
		seconds		
0	Control rate	0.09	188	100
	Thermode of 45°C. placed on S-A node	0.09	231	123
3.25	Thermode still on node	0.10	200	106
5.5	Thermode still on node	0.08	200	106
9.0	Thermode still on node	0.08	200	106
10.5	Thermode still on node	0.10	200	106
14.0	Thermode still on node	0.10	194	103
15.0	Thermode still on node	0.08	200	106
	After removal	0.08	167	89

a decrease to 228 after one minute's application of the thermode, and a drop to 125 after removal of the thermode. In experiment 9 the rate gradually decreased to normal as shown in table 1.

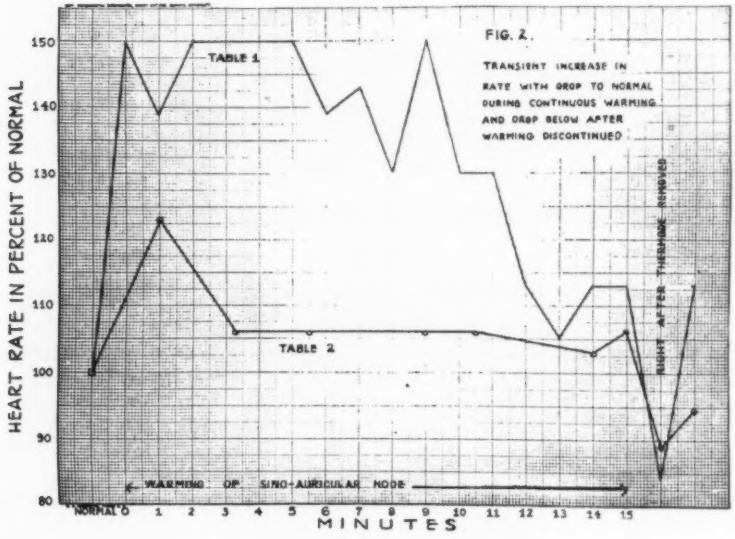

Fig. 2 summarizes tables 1 and 2

B. Long continued ether anesthesia: It has been our experience to have an animal on the operating table for many hours, and find the sino-auricular node just as responsive at the end as at the beginning of the experiment. Experiment 12 was done with that very purpose in mind. The reaction of the node was examined every 15 minutes for 6 hours under light ether anesthesia, and for $2\frac{1}{2}$ hours more at shorter intervals under both deep and light ether anesthesia. The initial heart rate of 176 per minute gradually dropped to 100 by the beginning of the eighth hour, and from then on it ranged from 84 to 94 per minute. Warming, however, always produced an increase in rate. At the beginning of the experiment the increases were 25 or more per cent, and at the end about 100 per cent.

C. Deep ether anesthesia: In advanced general anesthesia produced by ether, the heart is slowed, weakened and dilated. The auricles dilate considerably more than the ventricles. The effect of deep ether

anesthesia on the response of the sino-auricular node was studied by using the thermode for warming, by ethyl chloride spray for cooling the node, and with or without atropine administration.

The results show that no matter how intense the surgical anesthesia may be there is always an increase in rate produced by warming the sino-auricular node.

The following data are taken from the protocol of experiment 12.

TABLE 3

TRACING	TIME P. N.	PROCEDURE	BEATS PER MINUTE	INCREASE IN BEATS
35	3:06	Normal	103	
		Warming	200	97
36	3:07:08	Ether increased still more	105	
		Warming	176	71
37	3:10:30	Ether increased, more than in no. 36	100	
		Warming	146	46
38	3:11:30	Heart markedly weaker, ether increased	98	
		Warming	140	42
39	3:12:30	Heart weaker still, ether increased	91	
		Warming	125	34
40, 41	3:12:40	Ether shut off—air only given Heart becomes stronger		
42	3:22	Heart rate back to normal	103	
		Warming	207	104

In this case the increase on warming dropped from about 94 per cent to about 36 per cent with progressive etherization, and the return was to 101 per cent.

The effect produced on the form and rate of the heart chambers by increasing the amount of the ether is shown in figure 3. Variation in the ether is the only variable introduced.

The rate and size of beat is decreased, but the response to warming is the same in both as shown by the increased rate appearing toward the middle of both tracings.

Recovery from deep anesthesia is illustrated in figure 4.

Immediately before no. 1 is the end of a tracing taken with the animal thoroughly anesthetized. The numbers 1 to 7 show short strips

to note the gradual recovery upon withdrawal of the ether, and the beginning of the tracing immediately after no. 7 shows the complete recovery of the chambers. Figure 5 illustrates the same facts in the reverse order, from the normal to deep ether effects. These tracings

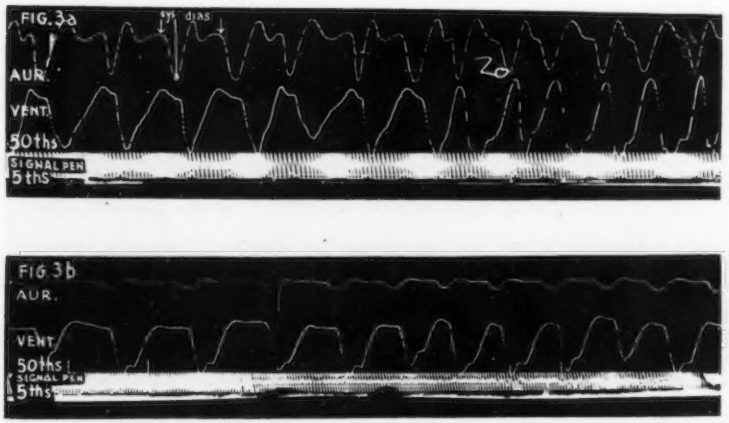

Fig. 3. Suspension tracings of right auricle and right ventricle in a dog. The down stroke indicates systole. A thermode (49°C.) was placed on the sino-auricular node. *a* shows the effect during light anesthesia, and *b* shows a similar substantial increase during deep anesthesia as shown by the weak beats and dilated chambers.

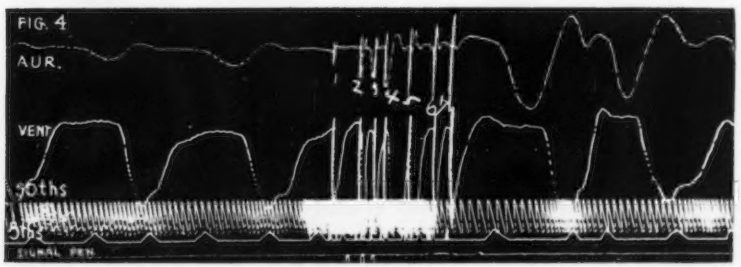

Fig. 4. Method of registration as in figures above. The heart is weakened by advanced ether anesthesia as shown before no. 1; no. 1 to no. 7 are short strips taken to show gradual recovery of the chambers to the adequate beat as shown after no. 7.

which are typical clearly demonstrate the fact that temperature effects were produced upon intact hearts in situ receiving blood containing a high percentage of ether.

In experiment 14 the normal rate of 167 per minute was increased on warming with 45°C. to 214. Ether was then given until the toxic stage appeared and although the auricle eventually became so weak (see fig. 5*b*) that on analysis of the curves the A_sV_s interval could not be determined, apparently because of no auricular contractility, warming still increased the cardiac rate from 111 to 207 per minute. This in

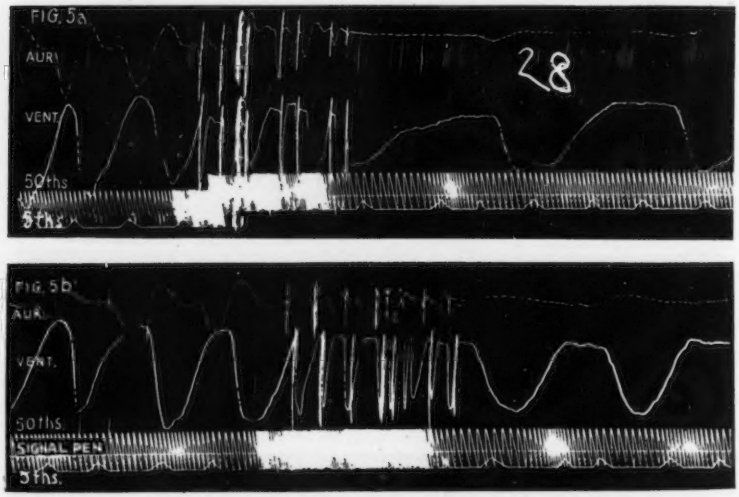

Fig. 5. Method of registration as in figure 3. Both *a* and *b* show transition in short strips of gradually weakening chambers by increasing the ether inhaled. Note the greater effect on the auricle.

point of fact is a greater percentage increase than in the normal heart. With removal of the ether, the normal rate and force of beat was resumed and a warming produced an increase of 167 to 240 beats per minute. This procedure was done at the very beginning of the experiment, and repeated thereafter with the same results.

A few experiments were performed showing the effects of cooling the node in an animal deeply anesthetized. In experiment 16 under light anesthesia the heart rate was increased from 207 to 214 on warming

before and after cooling. On cooling the rate dropped from 200 to 140 beats per minute. The ether was then given in excess and warming produced an increase although not as much as before, while cooling the node produced temporary stoppage of the whole heart. The heart recovered thereafter. If anything the heart responded more readily to the cooling under deep anesthesia. The following is taken from the protocol of another experiment, no. 17. Cooling here shows that the pacemaker was in the sino-auricular node in the deeply anesthetized animal.

TABLE 4

MINUTES	PROCEDURE	BEATS PER MINUTE	PER CENT OF NORMAL
0	Normal rate—Light ether anesthesia	162	100
	Warming S-A node with 55°C.	240	148
2		171	
	Ethyl chloride spray on S-A node	95	56
7	Ether given in excess		
8		158	
	Warming S-A node with 53°C.	214	135
9		176	
	Ethyl chloride on S-A node	122	69
$9\frac{3}{4}$	Ether removed	154	
13	Warming S-A node with 55°C.	250	162
$14\frac{3}{4}$		162	
	Ethyl chloride on S-A node	118	73

DISCUSSION

It has been the experience of the Wisconsin laboratory that the onset of negativity as recorded directly from the heart substance shows the sino-auricular node persisting as the primary pacemaker of the heart after long periods of ether anesthesia.

Although the temperature method is not as delicate as the electro-negative still for this investigation it was adequate as shown by the data and confirmatory of the latter method.

The experiment in which we kept an animal on the operating table for more than 8 hours is significant in that it shows that the primary cardiac pacemaker of the heart persisted throughout. This in spite of the exposure of the heart to the room atmosphere which inevitably cools the heart sufficiently to slow it somewhat, and the long period of exposure to a low concentration of ether in the blood stream. This in itself would seem to be sufficient to prove that ether is not markedly

toxic to the sino-auricular node. Corroborative evidence is found in the response of the node during deep ether anesthesia discussed below.

The reaction to long continued periods of warming is curious because it presents the possibility that there may be such a thing as fatigue of the sinus node with either a decrease in its automaticity or a shifting of the cardiac pacemaker. It has been shown that the pacemaker can change its location, and that when it does so it is found lower down in the coronary sinus or A–V node and bundle. When this occurs, the A_sV_s interval usually decreases. With high temperatures, as shown, the heart may become much slower after a transitory tachycardia. In some cases of clinical paroxysmal tachycardia (13) a similar condition prevails for a short period on recovery. The striking fact in our work is that a *persistent* experimental tachycardia is impossible by continuous warming of the sino-auricular node. Usually the sino-auricular node does not maintain its rate for more than a minute. Apparently the processes that produce initiation of the cardiac beat require something which rhythmically limits their speed normally to a set rate—an "habitual" rate. Bayliss mentions the views of Ringer and Howell on the cause of the heart beat which supposes that these nodes (S–A and A–V) discharge when they have stored up something to a sufficiently high degree, and that, after a discharge, they are incapable of further discharge until a fresh quantity has been formed. According to our results there can be only a temporary increase in the speed of these processes in the normal heart.

The data derived from animals in the toxic stage of ether anesthesia prove that the primary cardiac pacemaker persists no matter how much the contractility of the cardiac musculature has been decreased by the ether. It seems that in some of the experiments the property of contractility was dirempted from that of automaticity by the ether. For there were cases in which the auricle was greatly dilated and barely showed movement, if any at all, but the impulse was transmitted to the ventricle nevertheless. The same percentage increase in rate was shown by warming as in the lightly anesthetized animal.

It is possible that with the decrease in rate produced by ether the cardiac pacemaker in turn is located lower down in the specialized tissue. This phenomenon of removal has been shown to occur when, for example, the sino-auricular node was cooled or excised (14). On the other hand we have called attention (3) to the possibility that the response of a certain region may be the result of increase of its automaticity by the heat until it becomes the pacemaker, and does not

necessarily mean that it was the seat of impulse initiation before the heat was applied.

In reference to the above possibility, we have the following data which show that the pacemaker remained at its original site.

First, it was noted that on applying the heat the increase in rate occurred at once. There was no latent period. We have shown in a previous communication that such a latent period exists if the procedure is such as to increase the automaticity of a tissue sufficient to bring the pacemaker to that tissue. Or stating it in other words, if the pacemaker is in the tissue it responds at once to the warming.

Second, if the pacemaker occupies a particular position cooling will affect it at once, and will produce a slowing in the rate by either depressing the automaticity of that tissue or else driving the pacemaker to lower portions of the specialized tissue which in turn initiate slower cardiac rhythm. It is not conceivable that cooling tissue at a distance from the site of the pacemaker would influence the pacemaker. The possibilities of nerve influences have been discussed by us in our paper on the temperature method (3). We found no changes after atropine administration.

These last results on animals under general anesthesia are of importance in emphasizing the stability of the pacemaker in normal hearts. Further, that in the future a discussion of the pharmacologic action of drugs cannot ignore the fact that the specialized tissue of the heart is as much an entity as the heart muscle proper for the properties of the latter may be profoundly influenced with little or no effect produced on the former.

Conclusions for Series I, see summary at end of paper.

SERIES II. EXPERIMENTAL TACHYCARDIA

Instances of the experimental production of auricular flutter and tachycardia resembling that in man as well as other cardiac irregularities produced by warming the sino-auricular node are included in this series.

In Series I, data were submitted showing the results obtained by continuous warming of the node. A persistent tachycardia cannot be produced by warming the sino-auricular node for *long* periods in lightly anesthetized animals (cat and dog) whether they are atropinized or not. Instead, the rate slows after a transitory tachycardia down to a rate close to or less than normal, and upon removal of the thermode

the rate usually drops below the normal. These data will be submitted here in greater detail from a different viewpoint, rather as a study of the effects produced by warming than as the effects produced by the ether.

The clinical interest in cases of paroxysmal tachycardia as well as auricular flutter coupled with their unknown etiology furnished the stimulus for this study. Experimental production of these types of cardiac disorders was attempted by a method that would be fairly free from criticism and the peradventures of direct electrical stimulation of cardiac tissue which heretofore has been the commonest method employed. Lewis (15) has repeatedly emphasized the fact that the experimental side of paroxysmal tachycardia has remained practically untouched, and if such a tachycardia could be obtained with regularity, a study of it, at whatever level it might have its origin, would materially assist us in understanding the factors which operate in the production of the two main types of paroxysmal tachycardia known to occur in the human subject. Of course, not only the origin or production of the cardiac disorder, but its mechanism as well, are of interest.

Heretofore, on the basis of electrocardiographic studies the assumption has been that paroxysmal tachycardia impulses usually originate at points removed from the normal pacemaker. In this paper data are presented showing the heart picture during *short periods* of warming the sino-auricular node, the normal site of the cardiac pacemaker, as well as *long continuous periods* of warming, in other words, a nomotopic or homogenetic paroxysmal tachycardia, and auricular flutter.

The method of direct electrical stimulation of the cardiac tissue and the consequent phenomena—tachycardia, flutter and fibrillation—has been reviewed by Hirschfelder (16). It is well known that handling or just touching the normally beating heart may set free short periods of tachycardia. Other means of mechanical interference have done the same, but tachycardia is not an invariable accompaniment of such procedure. This detracts from the mechanical method. Stimulation of the cardiac nerves, with or without the simultaneous administration of drugs, occasionally produces paroxysmal tachycardia (17). A more radical method is that of ligation of the coronary arteries (15) with the intention of inducing tachycardia as done by Lewis. Porter (18) ligated the coronaries with another purpose in mind, and gives no data concerning increased rates. In seven of Lewis's seventeen experiments on dogs nothing occurred. In the other ten, tachycardia

periods, auricular, nodal and ventricular, of a few seconds to 35 minutes, appeared 15 to 126 minutes after ligation. An average normal rate of 126 per minute was increased to 253. Vagal stimulation affected the auricle only. Tachycardia occurred even after section of both vago-sympathetic nerves. No transitional rates appeared at the onset or termination of a period. Electrocardiographic curves were taken.

More recently F. M. Smith (19) studied the effect of ligation of definite branches of the coronary arteries in sixty-six dogs. Electrocardiograms were taken. He noted the rather common occurrence of paroxysmal tachycardia. Such work is satisfactory because it is done aseptically, and the percentage of recovery from the operative procedure was fairly good, varying from 9 to 57 per cent, depending upon the branch ligatured. Even this excellent aseptic method is at fault, however, in that there can be no previous knowledge of the type of disorder bound to occur, and when for instance paroxysmal tachycardia does occur, it may have associated with it other irregularities that disturb the issue.

In addition to the methods given above, there is another interesting one in which there is voluntary production of paroxysmal tachycardia (20) in man, and similar control of its offset. These probably have a neurogenic origin. Obviously extensive research cannot be done on such a rare human subject.

All treatises on cardiac diseases postulate a paroxysmal tachycardia of nomotropic origin, but as yet very few cases, either clinical or experimental, have been described. By our method of warming the sinus region three anatomic groups may be affected—intracardiac vagus, intracardiac accelerators, and sinus node. A tachycardia may result by *a*, depression of the vagal endings, and this would most likely not occur by moderate warming, if anything, an initial slowing might be expected; *b*, by increasing the irritability of the accelerator mechanism; and *c*, by increasing the rhythmicity of the sinus node. If a tachycardia occurs even after the administration of atropine, then the vagus mechanism could be ruled out. It is conceivable that increasing the efficiency of the accelerator mechanism would produce a tachycardia which could not however be designated as paroxysmal tachycardia, but rather as simple tachycardia because there would only be a moderate increase in rate. The probabilities are that the increased efficiency of both the vagus and accelerator mechanisms would about counterbalance each other and the net result would be an unchanged rate. Moorhouse, working with excised sinus node strips, never observed an initial slow-

ing with increase in temperature. This then leaves only the sinus node proper as the chief structure influenced. In such a case we have a "nomotropic" tachycardia which can be made "paroxysmal" in type by *intermittent* periods of warming. This we have done repeatedly in experiments in which there has been no atropine administered as well as in those in which atropine was given in dose sufficient to rule out the vagus.

The characteristics of such a tachycardia are first, an immediate increase in rate, clinically designated as extreme abrupt acceleration or onset. This sudden onset occurs every time the sino-auricular node is touched by the thermode, and kept there.

Whether a certain percentage increase is such as to place the case in the paroxysmal tachycardia group using the rate as a criterion will depend to a great extent upon the tonus of the vagal mechanism. In etherized animals the heart rate is usually above normal (21) unless the ether anesthesia is light and not sufficient to reduce vagal tone.

With open chest work under anesthesia, and with no administration of morphine, many hearts have a rate beyond the normal range of 78 to 140 which has been found by Gasser and Meek (22) to be the average resting pulse in intact and unanesthetized dogs. They have shown that complete release of the vagus by atropine administration will produce rates of 188 to 232 in the same animals. We have had initial rates of 115 to 143 (7 out of 17 experiments), and in some of these we have practically doubled the rates by warming. In the others with a rapid rate at the start suitable warming increased it to a point which was surely double or more than double that normally existing in that animal. It is conceivable that in these latter cases the same cardiodynamic relations prevailed when the rate was made excessive as in those where there was an evident doubling in the rate. This will be explained a little later.

Rarely, the fast rate was preceded by one or several extra-systoles. In experiment 16 there was an instance of two very slow beats initiated by the warming.

The effect on the A_sV_s interval has already been presented under Series I.

Normal sequence of auricles and ventricles prevailed during the periods of tachycardia in all the experiments. A 1 to 1 rhythm is also the rule in clinical paroxysmal tachycardia. Exceptionally, instances of independent auricular extra-systoles, independent ventricular extra-systoles, auriculo-ventricular extra-systoles, nodal beats, partial auriculo-ventricular blocking, and probably sino-auricular block occurred.

In these cases of short periods of warming the rate remained high and fairly constant. They did not show the variations described by us during periods of warming of comparatively long duration (see Series I).

Postparoxysmal phenomena are rare occurrences. In experiments 3 and 16 a long pause is seen. In experiment 9 a short period of auriculo-ventricular block occurred. The general rule was an immediate resumption of normal rate after short periods of warming. We have already shown this response to be characteristic in localization of a *primary* cardiac pacemaker when warming the sino-auricular node. Clinically, a slow sinus rate as a sequence to paroxysms has been noted as an inconstant feature in some cases. In others it has been a constant feature. Its presence is apparently an indication of the ectopic character of the new rhythm.

With our method it is probably impossible for any statment to be made on the duration of ventricular systole or more specifically, systolic ejection. We expect to report further on the mechanism of paroxysmal tachycardia. It is well known that the mechanical efficiency of the heart when beating very rapidly can be impaired even though the beats are regular and coördinate by reducing the amount of blood pumped out per minute. On the other hand it is well known that a considerable increase in heart-rate may occur without apparently reducing the filling or the systolic ejection of the ventricles seriously. It has been shown by Henderson that the cardiac output in dogs is reduced when the rate is maintained above 240 per minute. This rate is about twice that normally prevailing in the dog and is the rate predominating in our experiments as a result of warming the sino-auricular node. Wiggers and Katz (23) have also called attention recently to the marked effect on systole during rapid rates produced by accelerator stimulation. Clinically it has also been shown that rates double those normally found in man if continued for some time will eventually produce signs of impaired filling and cardiac output. The systolic arterial pressure falls; the venous pressure rises, and venous engorgement appears—cyanosis, liver enlargement, laryngeal edema, increased cardiac dulness, râles, dyspnea, etc. In man the tachycardia need not necessarily be one prolonged attack to produce evidences of heart deficiency. Repeated attacks occurring close together lasting only a few beats or a few minutes are sufficient to cause impaired cardiac efficiency in man, and it is practically this type which we produced in our experimental animals. They bear a close resemblance to the following cases: Lewis (13) reports a case in man which from the

standpoint of frequent recurrence of tachycardia periods shows remarkable similarity with our experiments. He gives a diagram of 54 paroxysms in 1 hour 49 minutes. "The case is remarkable for the persistent recurrence of short attacks." The longest paroxysm recorded was 7 minutes; those exceeding a minute were rare; and the shortest period consisted of 4 rapid beats. Hart (24) published some excellent curves of paroxysms lasting from a few seconds to several minutes. An unusual type of auricular paroxysmal tachycardia was reported by White (25) in which the paroxysms rarely lasted more than 10 seconds each. The electrocardiogram in this case showed a P wave that was normal or close to normal. In other words it is probably sino-auricular paroxysmal tachycardia. Galli (26) just recently reported observations on sino-auricular paroxysmal tachycardia.

Concerning the efficiency of the extracardiac nerves during periods of tachycardia induced by warming the sino-auricular node Ganter and Zahn and others have presented data attempting to localize the distribution of the nerves. This phase of the matter will be reported later.

The results given above as derived from our own data are substantially those that could be derived from the investigations of Ganter and Zahn and others but not reported by them. They, however, were primarily interested in the responsiveness of the sinus node region and other specialized cardiac tissue rather than in any similarity to a functional cardiac disorder like paroxysmal tachycardia.

We propose to continue this study by noting the mechanical effect on the circulation of such a tachycardia, because regardless of the site of its origin a tachycardia means as a rule an accelerated action of the ventricles. It is this accelerated action of the ventricles upon which the clinical picture depends.

See summary on Series II below.

SERIES III. EXPERIMENTAL AURICULAR FLUTTER

Clinically, auricular flutter is a frank marked increase in the rate of the auricles with a simultaneous slower rate in the ventricles which is usually one-half to one-fourth that of the auricles. In other words, it is an auricular tachycardia associated usually with partial block and a rapid ventricle. Some cardiac pathologists arbitrarily designate a case in man as flutter when the auricles beat about 300 per minute, rapidly and rhythmically, with *a*, normal ventricular sequence, or *b*, partial

block, or *c*, ventricular irregularity in rhythm and in size of contraction. Generally, it is a uniform and established condition although cases have been described in which the attacks are paroxysmal. It is believed that the condition is almost always ectopic in origin. Blackford and Willius (27) have recently given an excellent review of auricular flutter.

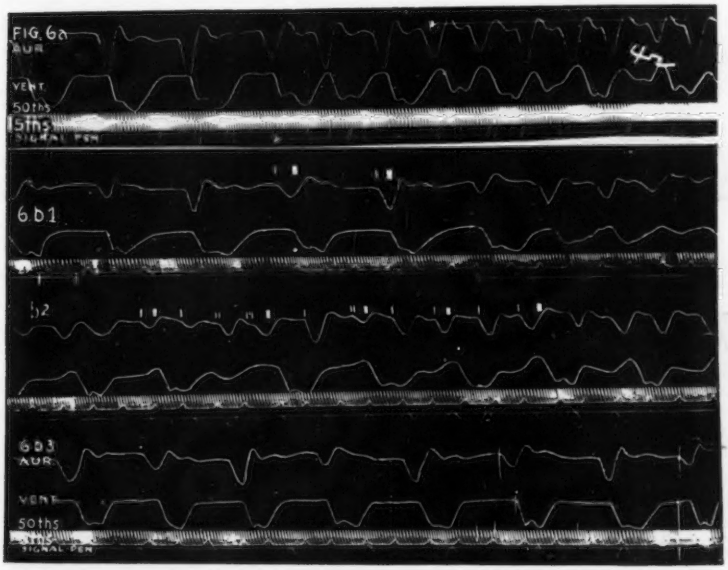

Fig. 6. Method of recording as in figure 3. Experiment 12. *a* shows the increased rate of the chambers by warming the sino-auricular node with normal 1:1 sequence. Tracing taken earlier in the experiment before the occurrence of the events shown in 6*b2*. *b1*, *2*, *3*, are strips taken before, during and after warming of the sino-auricular node. Both before, *b1*, and after warming, *b3*, normal 1:1 sequence is shown with rates of 84 and 90 per minute respectively. During warming, *b2*, the auricle practically doubles its rate, in one case 3:1 is seen, while the ventricle continues at practically its original normal rate.

Experimentally, with an initial and relatively slow rate, a condition of reduced conductivity of the A–V node and bundle may be present or induced, and the production concurrently of an increase in the irritability of the sino-auricular node so as to increase its rate of impulse formation may give a condition which simulates auricular flutter.

The initial slow rate may be suggestive of sino-auricular block. If, however, the rate developed, as for example by warming, is not excessive it remains practically in the field of partial heart block, and it is necessary to increase the temperature in the thermode which is applied to the sino-auricular node to produce a condition which might really be called experimental auricular flutter. We suggest such a method to study the mechanical efficiency of the circulation in flutter

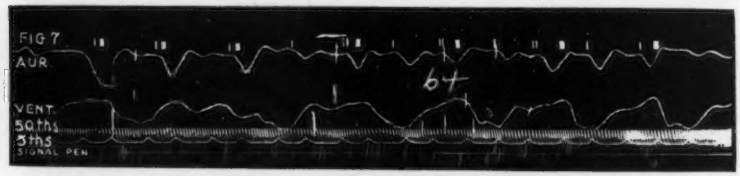

Fig. 7. Method of registration as in figure 3. This tracing shows transition from a normal 1:1 rhythm to a 2:1 rhythm.

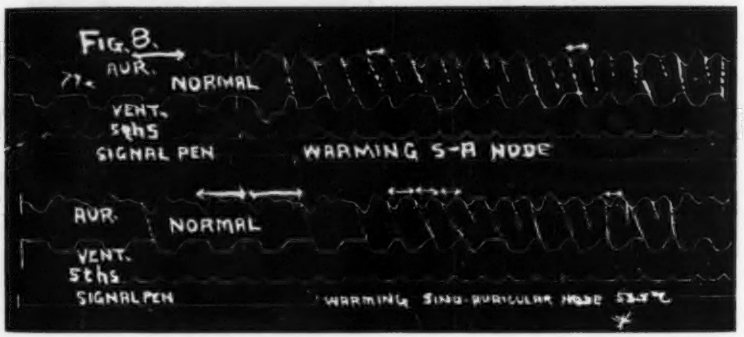

Fig. 8

because the methods heretofore used such as mild faradization (MacWilliam, 1887; Eyster and Swarthout, 1920 (28)), the injection of glyoxylic acid (Lewis, 1912–1913), ligation of the coronaries (Hirschfelder, 1908), and the injection of alpha-iodin (Kendall, Blackford, Willius, 1915) are inconstant, uncontrollable, and usually accompanied by other forms of abnormal cardiac rhythm.

Figures 6*b* and 7 illustrate moderate increases in auricular rate with a ventricular rate which is about one-half that of the auricles.

In one instance the auricular rate rose to 200 from a norm of 103, while the ventricle stayed at 100 per minute.

In another instance the auricular rate rose to 188 from a normal of 84, with ventricle at 94 per minute during the increase. The A_sV_s interval during the increased rate is either the same as normal or in some cases it is increased 0.03 to 0.07 second above normal.

It was stated above that the slow rate may have been suggestive of sino-auricular block or regular recurrence of dropped beats. One reason that may be given is that a tracing taken between those shown (fig. 6*b* and fig. 7) shows that a 1:1 rhythm can still be produced by warming the sino-auricular node.

In figure 8 taken from experiment 4 two curves are shown in which warming of the sino-auricular node produced a 300 per minute increase in the rate of the auricle with a slower rate of the ventricle. The A_sV_s interval shows the presence of dissociation.

Sino-auricular heart block. The criterion for partial sino-auricular heart block in the mammalian or human heart is the occurrence of dropped beats, auricular and ventricular, in an otherwise regular auricular rhythm.

Delayed conduction from the S–A node to the auricle may probably manifest itself in a splitting of the P wave on the electrocardiogram, or in an increase in the P R interval which in a suspension record of the auricle taken simultaneously would be shown by a longer interval between the beginning of the P wave and the beginning of auricular systole. In the suspension tracing itself the cycle would be longer than normal. Sino-auricular heart block and its criteria from both the clinical and experimental standpoint have been reviewed by Eyster and Meek (29), Eyster and Evans (30) and Lewis (31).

In experiments 3 and 8 the suspension curves of both auricle and ventricle showed occasional dropped beats as indicated by an approximate halving of the otherwise regular auricular rate made rapid by warming. Such failures of the auricle to contract with simultaneous silence of the ventricle may be due to a number of causes among which are *1*, weakness of the auricular muscle; *2*, inadequacy of the stimulus; *3*, blocking of the cardiac impulse between its point of origin and the auricle; *4*, stimulation of the vagus; and *5*, failure of the pacemaker to generate excitations at certain periods. Our method precluded data which could give a direct indication of the activity of the sinus region, and therefore the summary derived from the data for postulating instances of sino-auricular heart block will differ considerably from cases in which the electronegative method was used.

Table 5 gives the data showing length of beat in seconds, the A_sV_s interval, the rate per minute, the procedure, and increases or decreases before and after the long cycles. Figure 9 shows the tracing from which the data designated in table 5, as experiment 8, no. 5, was taken.

TABLE 5

Sino-auricular heart block

EXPERIMENT	ITEM	PROCEDURE	A_sV_s	AURICULAR BEAT	VARIATIONS	RATE PER MINUTE
			seconds	*seconds*	*seconds*	
8, no. 5	1	Before warming S-A node	0.080	0.52		115
	2	Before warming S-A node	0.100	0.50		120
	3	Beginning warming S-A node	0.080	**0.30**	0.00	200
	4	During warming, later	0.100	**0.34**	+0.04	176
	5	During warming next beat	0.120	**0.32**	+0.02	188
	6	Last beat during warming	0.110	**0.32**	+0.02	188
	7	Next beat after warming	0.110	**0.66**		91
	8	Following beat	0.110	**0.38**	+0.08	158
	9	Following beat	0.120	0.40	+0.10	150
	10	Following beat	0.120	0.41	+0.11	146
3, no. 3	11	Before warming S-A node	0.094	0.46		130
	12	Before warming S-A node	0.090	0.46		130
	13	During warming S-A node	0.095	**0.24**	0.00	250
	14	During warming next beat	0.076	**0.22**	−0.02	273
	15	During warming next beat	0.085	**0.26**	+0.02	230
	16	During warming next beat	0.095	**0.57**		105
	17	During warming next beat	0.114	**0.43**	+0.19	140
	18	During warming next beat	0.087	**0.39**	+0.15	114
	19	During warming next beat	0.086	0.41	+0.17	146
	20	After warming	0.104	0.49		122
3, no. 7	21	Before warming S-A node	0.126	0.37		162
	22	During warming, at start	0.090	**0.25**	0.00	240
	23	During warming	0.103	**0.27**	+0.02	220
	24	During warming next beat	0.125	**0.65**		93
	25	During warming next beat	0.097	**0.49**	+0.24	123
	26	During warming next beat	0.098	**0.43**	+0.18	141
	27	After warming	0.085	0.34		175

In conceiving the conditions existing in sino-auricular block it is usually assumed that there is a conduction delay or retardation of the impulse initiated in the sinus node on its way to both the auricle and auriculo-ventricular node and bundle, so as to lengthen the sino-right

auricle interval, and sino-ventricular interval. Eyster and Meek note that the conduction period gradually lengthens in their electronegative tracings until complete failure of conduction occurs. When this condition of block merges into complete stoppage of the sinus impulse so that it is not conveyed to infrasinus regions, then one or more beats will drop out. In suspension tracings the dropped beats are probably preceded and followed by a lengthened sino-auricular interval as indicated by a progressive lengthening of the cycles before the dropped beat occurs. Likewise, subsequent to the dropped beat the cycles progressively lengthen. This can be seen in table 5. The beats occurring before the absent beat or beats have increased 0.02 to 0.04 second (7 to 13 per cent) while the cycles after the block are 0.08 to 0.24 second longer. This makes the absent cycle a little more than 2 or 3 times

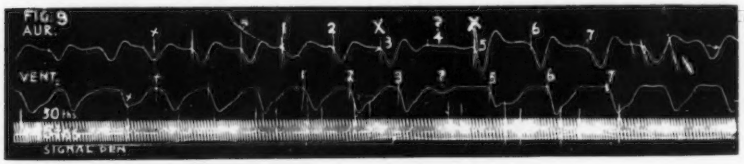

Fig. 9. Method of recording as in figure 3. At *xx* a long cycle is shown which is practically twice the length of the previous cycles. There is evidently a dropped beat indicating 2:1 sino-auricular block. See figure 1 for an example of a 3:1 sino-auricular block.

the length of the previous cycles, and less than 2 or 3 times the length of the cycles after the cardiac silence. That the beats in the slow rhythm do not arise in the A-V node is indicated by the fact that they have an approximately normal A_sV_s interval.

SUMMARY

1. The primary pacemaker of the heart maintains its normal locus in both light and deeply etherized animals even to the toxic stage with auricular dilatation. This is true also when the heart is atropinized. Local warming and cooling by a specially devised thermode was the method used.

2. A persistent tachycardia cannot be produced by warming the sino-auricular node for long continuous periods in lightly anesthetized animals (cat and dog) whether atropinized or not. Instead the rate slows after a transitory tachycardia to a rate close to or less than normal, and upon removal of the thermode the rate usually drops below normal temporarily.

3. A tachycardia of nomotropic origin with short periods of sudden onset and termination resembling certain clinical cases of paroxysmal tachycardia may be produced experimentally.

4. Occasionally, an experimental auricular flutter can be induced by warming the sino-auricular node.

5. Instances of probable sino-auricular heart block experimentally induced by warming are described.

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V. EXPERIMENTAL PRODUCTION OF VENTRICULAR FIBRILLATION BY LOCALIZED WARMING OF CARDIAC TISSUE

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The importance of ventricular fibrillation clinically has been recently emphasized by Lewis (1), Levy (2) and others, although at present unfortunately it is a terminal phenomenon. Various methods of experimental production of ventricular fibrillation, therefore, are of interest to cardiac pathologists, and a method acquires significance if it is possible to repeatedly produce ventricular fibrillation in the same animal. This we have done in advanced digitalization of the heart by warming rather limited portions of a ventricle or the coronary sinus.

Method. The method used has been described in the preceding paper (3). In 15 experiments digitalis was given intravenously to 7 dogs and 4 cats, strophanthin was administered by the same route to 3 dogs, and 1 dog received neither of the drugs. The regions warmed were small restricted areas on the right and left ventricles, the apex and the coronary sinus.

Results. While studying the response of the specialized tissue after digitalis administration we believed that localized warming would be effective during the toxic stage of the digitalis action when the ventricles are known to be beating independently of the auricles. It is then that increased ventricular irritability manifests itself among other ways in marked increase in ventricular rate in the well-nourished heart, and the latter could be influenced in regard to rate.

Warming the apex in four different experiments resulted in an increased response of the ventricle in three out of the four times. Warming of the apex in the heart not treated with digitalis was ineffective. Figure 1 shows the ventricular rate before warming to be 150 per minute, while during warming it is 225 per minute. This irregular tachycardia is typical as one of the premonitory signs of ventricular

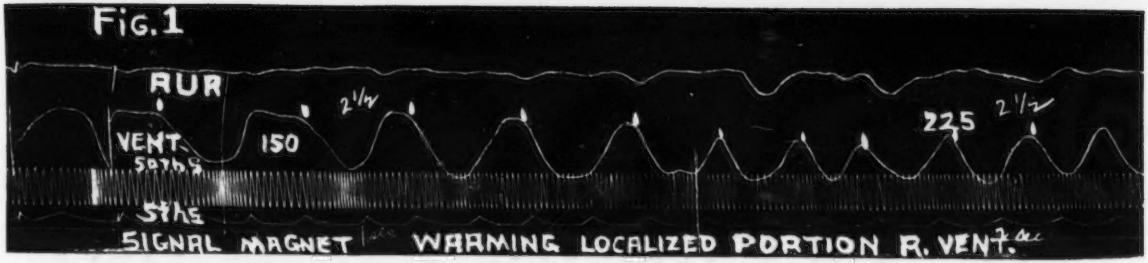

Fig. 1

Fig. 2

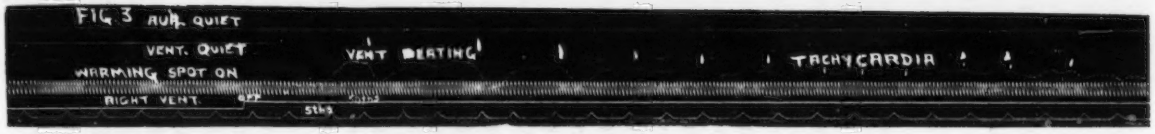

Fig. 3

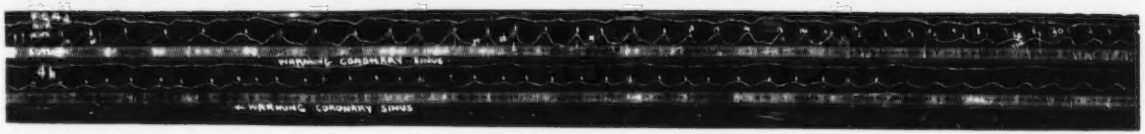

Fig. 4

fibrillation. The next stage, consisting of ineffectual fast beats following the tachycardia, which finally is submerged into fibrillation, is seen in figure 2. This occurred during warming of the apex also.

Applying heat to the right ventricle produced the tachycardia with prefibrillatory characteristics in three out of seven experiments. In experiment 7 (fig. 3), a very striking phenomenon is seen. Here both heart chambers are shown to be quiescent. Immediately at the end of a period of warming the ventricle began beating slowly, then increased rapidly in rate. The auricle throughout this period was paralyzed.

The results with the left ventricle were unsatisfactory as compared with those obtained above. Warming produced an increase in only one out of four experiments.

In five out of nine experiments warming of the coronary sinus was followed by a marked increase in rate. In experiment 4 (fig. 4), the ventricle was beating approximately 170 times a minute. This was increased to about 230 times a minute during the warming of the coronary sinus. In figure 4*b* will be noted the weakening of the beats during the same procedure. Later in the same experiment with continued warming the rapid irregular tachycardia gradually merged into coarse undulations of the ventricle which eventually were less extensive and finally terminated in minute, widespread twitchings of the ventricular muscle. Then, upon removal of the thermode, the reverse occurred. This sequence of events was also produced by warming the right ventricle in other experiments.

Discussion. In the toxic stage of digitalis poisoning it is well known that the ventricles assume a rhythm of their own which is ectopic in origin. It is believed that these ectopic beats arise usually from many foci either in the auriculo-ventricular bundle and its branches or in the cardiac muscle itself. It has been shown by the direct lead method or in other words by the taking of electrograms with the galvanometer that during the late toxic stages of digitalis administration the ventricles are beating independently. Shigeru Sakai (4) and others have demonstrated the beats to be ectopic in origin with this method. With the tendency of the ventricle to assume independent automaticity, localized warming of the musculature would lead to a marked increase in the irritability of the part warmed. This spot in turn will assume the pace-making function of the ventricle as we have shown, and continue as such, even to the onset of fibrillation, if warming is persisted in. Upon cessation of the warming the true fibrillation

translates itself into feeble irregular contractions, which in turn go over into coarse undulations, finally ending in the original beat.

Our observations and tracings show that ventricular fibrillation does not come on, in its fully developed form, suddenly. It is preceded by a definite series of phenomena as indicated above. This fact has also been observed by Lewis, McWilliam, Robinson (5) and others.

SUMMARY

A method is presented by which it is possible to obtain a heart in which localized warming will initiate ventricular fibrillation. This can be done repeatedly if the heart is previously digitalized and tested during the toxic stage when the ventricles are beating independently.

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